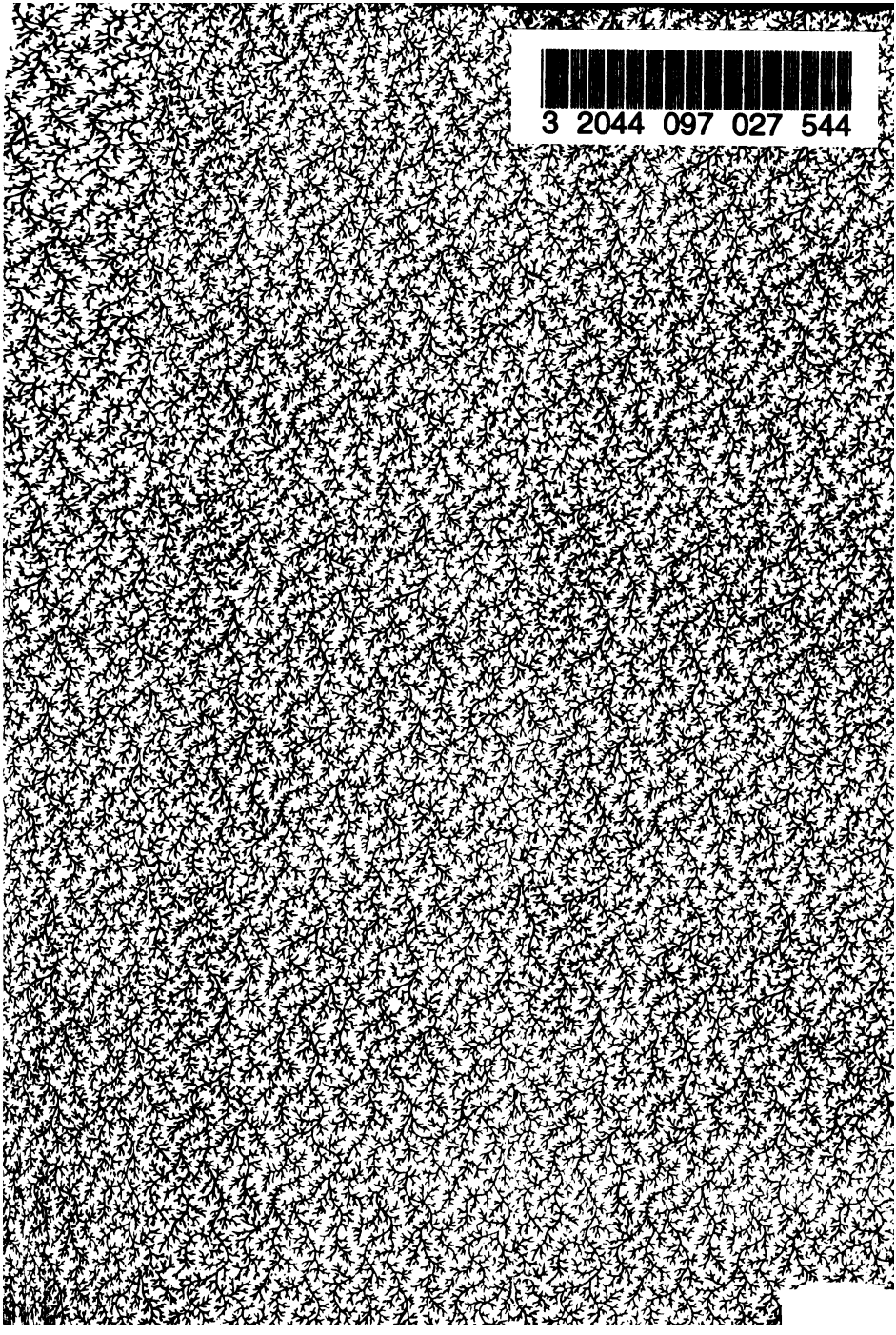

LABORATORY COURSE

IN

INVERTEBRATE ZOÖLOGY

BY

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PREFACE.

In the preparation of this book the author has had in mind the requirements of a class of students who are pursuing a course of laboratory work in Invertebrate Zoölogy. An effort has been made to direct the work, without, at the same time, actually *telling* the student all that there is to be learned from the specimen. It is taken for granted that an instructor is present to assist when there is trouble, and to demonstrate many things that written descriptions might only render more confusing.

The animals that have been selected are not always the most typical, but they are generally forms that can easily be secured and preserved.

In the Appendix a few words have been given regarding laboratory methods, etc. It has not been thought advisable to introduce any considerable treatment of the microscope as an optical instrument, nor have the modern

and valuable methods of microscopical research been more than outlined. The former will be found in numerous text-books; for the latter, it is expected that the student will consult Whitman's "Methods of Microscopical Anatomy and Embryology" and Lee's "Microtomists' Vade-Mecum."

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March, 1892.

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INVERTEBRATE ZOÖLOGY.

A RHIZOPOD PROTOZOAN

(*Amœba sp.*).

PLACE a drop of water that is known to contain *Amœbæ* upon a slide and cover with a thin cover-glass. Having found an *Amœba*, note the following points:

The outline is irregular, and, if the animal is active, constantly changing. The ray-like prolongations of the body are called *pseudopodia*. Are the pseudopodia simple, or are they branched? How many are there?

The body of the animal is apparently made up of two portions,—an inner granular portion, the *endosarc*, and an outer clear layer, the *ectosarc*. Is there endosarc in the pseudopodia? Careful observation will reveal the presence of a circulation of the granules of the endosarc.

Examining the endosarc with more care, it will be found to contain *food vacuoles*, a *vesicle*, and a *nucleus*.

The *food vacuoles* are small, generally spherical bodies, in color depending on the food that has been ingested. Around certain vacuoles a clear enveloping space may be noted.

The *nucleus*, not often clearly seen in the living animal, may be definitely demonstrated by adding a drop of one per

cent acetic acid to the water holding the *Amœba.* The acid kills the animal and colors the nucleus light brown.

Make drawings illustrating the points above considered.

Observe a living specimen as it crawls about, and see if it advances one portion of its body in preference to another. Jar the slide. Is the animal sensitive? If possible, observe the animal feed. It may be fed with powdered carmine, indigo, or Bismarck brown.

At times, by a process of *fission*, or *self-division*, the *Amœba* divides into two smaller *Amœbæ*, which may in turn again divide. This method of multiplication is *asexual.*

A CILIATE PROTOZOAN

(*Paramœcium caudatum*).

PREPARE slides in the same way as was directed for *Amœba*. If the animals have been fed for some hours with carmine or Bismarck brown, the internal structure will often be more clearly shown.

Under the compound microscope the *Paramœcia* are seen to glide rapidly about, lashing their way through the water by means of innumerable *cilia*. Have they an *anterior* and a *posterior* end? Find an animal that is quiet, and see if you can distinguish between an external *membrane* and the enclosed granular *cytoplasm*.

Are all the cilia of about the same length? Note their arrangement along the edges of the opening that leads into the animal from the side. This opening leads into the *vestibule*.

The vestibule is the ciliated passage down which food is forced on its way to the cytoplasm. Is there a definite flowing of the cytoplasm? Are the contained food vacuoles different from those of the *Amœba?* The food-balls, after losing their nutritious portions, are ejected from the body at a point, the *anus*, situated a little way posterior to the vestibule.

Do you find *pulsating vacuoles?* How many? What is the outline in diastole? In systole?

Use dilute acetic acid, and note the form and arrangement of the *trichocysts*, defensive bodies comparable to the nematocysts of Cœlenterates.

A ciliate infusorian contains a *macronucleus* and one or more *micronuclei*. The former is a comparatively large body and can readily be observed by the use of dilute acetic acid. The micronuclei are smaller and, in some cases, difficult to find.

Paramœcia are often to be observed attached in pairs by their vestibular surfaces, in process of *conjugation*. After remaining attached for some little time, the two become free and undergo division by *fission*.[1]

Make a drawing of *Paramœcium*, naming the parts.

[1] Conjugation is completed by disappearance of the macronuclei, which are supposed to be nutritive bodies for use during this period of inactivity. The micronuclei, during conjugation, are actively dividing, and previous to separation one of these divisions in each organism passes over into the body of the other.

AN ASCON SPONGE

(*Leucosolenia sp.*).

General Anatomy. — Specimens of *Leucosolenia* may be abundantly found along the New England shores, attached to rocks, seaweed, and submerged wood-work near the low-tide mark. The specimens submitted for examination will be found to vary considerably in size and to not infrequently occur in little groups or clusters.[1]

Examine the specimens with a hand lens and note the following: —

Each *sponge body* is cylindrical and is attached by one end, the *proximal*, to some foreign support, while its *free* end presents a large opening, the "*osculum*." Press the sponge, and note that its *wall* is flexible. The larger sponges bear *buds* or *branches*. Do the buds or branches have oscula? Do the walls of the sponge become in any way different as they approach the rim of the osculum?

Carefully lay open the sponge its entire length, cutting from the osculum to the base. The simple *central cavity* or *cloacal chamber* is thus exposed. Does the cloacal chamber communicate with the smaller cloacal chambers of the buds?

Place one half of the sponge where it will quickly dry, and the other half, after washing it for a moment in water, place in alum or borax carmine, where it may remain from one to

[1] Specimens preserved in alcohol are more desirable for laboratory work than the living animals.

several hours. Utilize the time while the piece is drying, by drawing a cluster of the sponges (natural size, and also somewhat enlarged), illustrating such characters as have been considered.

Place the now dry piece of sponge upon a clean slide and examine the cloacal surface, using a low-power objective. The sponge appears to be made up of a multitude of needle-like bodies or *spicules*. The spicules are clearly of two kinds, *radiate* and *linear*. The radiate spicules form a somewhat regular lattice, each spicule producing its three longer rays parallel to the wall of the cloacal chamber. A fourth and shorter ray extends, from the common point of origin of the three longer, perpendicularly into the cloacal chamber and, as the sponge now lies, towards the eye of the observer, as may be demonstrated by the use of the fine adjustment. The *linear* or *biradiate* spicules are somewhat curved. Do they lie in a plane mainly without or within the "*quadriradiate spicules*"?

Make drawings of the spicules, giving them their relative dimensions.

Remove the specimen from the stain, wash it for a moment in water and then carry it through grades of alcohol to absolute. Clear in clove-oil and mount in balsam, cloacal surface uppermost. (Do not allow the cover-glass to rest too heavily upon the specimen.) Examine under compound microscope.

The spicules will now be found to lie below (outside of) a layer of small cells, the *endoderm*, the nuclei of which are clearly defined. The endoderm entirely covers the inner surface of the cloacal chamber, and its cells, during life, are provided with *flagella* and "*collars*," the activity of the former producing a current of water, which enters the chamber

through numerous *pores* and leaves through the osculum. The pores are easily determined, in the preparation under consideration, as small round openings free from nuclei and surrounded by the rays of the spicules.

Outside the endoderm (below in the present specimen), and surrounding the spicules, are the *mesoderm cells*. The mesoderm cells are not arranged as a single layer, but occur irregularly through the mass of spicules. Their nuclei are often seen as granular bodies along the rays of the spicules, and their protoplasm appears as a faintly stained reticulum. While the endoderm cells have a definite outline, the mesoderm cells are irregular and *multipolar*. The spicular skeleton is formed by the activity of the mesoderm cells.

Still a third layer of cells, the *ectoderm*, has been described. Its cells form a very thin tissue, easily destroyed, and too difficult of demonstration to be here considered. It covers the outer surface of the animal, and is probably somewhat soiled with foreign matter.

Make a drawing illustrating the points above mentioned.

Reproduction. — Many of the larger specimens prepared and mounted as above directed will be found to bear *reproductive cells*. These cells appear as large, deeply stained bodies resting in the mesoderm and covered by the endoderm. They are of two kinds, the male and the female. The male cells or *spermatozoa* occur in *spheres*, each sphere containing several hundred *sperm-cells*. The *nuclei* of the sperm-cells give to the surface of the sphere a peculiar dotted appearance, and are considerably smaller than the entodermal nuclei. The sperm-spheres, however, are large, their diameter being about one-third the length of a long ray of a quadriradiate spicule.

The *ova* or *egg-cells*, though often as large as the sperm-spheres, are provided with only a single large nucleus. In this latter may rest a *nucleolus*. The egg-cells are often amœboid in form. They are seldom spherical.

Make drawings of ova and sperm-spheres.

Segmenting eggs are not infrequently found. Such eggs often have a clear space around them and between their several segments or *blastomeres*. Two- and four-celled stages are most abundant. Are all the blastomeres provided with nuclei? Can you find an unsegmented ovum that has two nuclei?

If a cluster of clean sponges be placed in two per cent chromic acid for a day and then carefully washed, stained, imbedded in celloidin and cut transversely, the arrangement of the cell-layers and the structure of the segmenting ova will be more clearly shown. The endoderm will be found to consist of closely appressed, somewhat *columnar* cells, forming a layer considerably thinner than the outer, less distinct mesoderm. The delicate ectoderm will probably be destroyed.

The spicules have been dissolved by the chromic acid, but the cells once surrounding them are now seen to be quite irregular in outline and to hold in their meshes the ova and the sperm-spheres.

Make a drawing of the transverse section.

A SYCON SPONGE

(*Grantia sp.*).

General Anatomy. — Simple sponges, which have been described under the name *Grantia*, are found associated with *Leucosolenia* along the Atlantic coast. Specimens vary considerably in size, the larger sometimes reaching one inch in length.

Examine a dry specimen with a lens and note the general outline of the *body*, the somewhat expanded *base*, the terminal *osculum*, surrounded by a *funnel* of spicules, and the numerous *incurrent pores*, distributed over the external surface of the body, and more or less hidden by the numerous spicules. Are there *buds* attached to the sponge body?

Make a drawing illustrating the above points of the external anatomy.

With a sharp scalpel open the sponge by making a longitudinal stroke from base to osculum. The central cavity or *cloaca* will be exposed, and its walls will be found pierced with numerous openings, the *gastric ostia*. Note that the cut edges, much thicker than in *Leucosolenia*, are traversed by parallel tubes, those entering the central chamber through the gastric ostia being called *radial canals*, while the shorter, which enter from without through the incurrent pores, are called the *incurrent canals*. The incurrent canals communicate with the radial canals through certain small openings

which cannot be seen at present. Do you find anything that might strain the water as it enters the incurrent canals? Do the buds communicate freely with the cloaca of the parent?

Examine with lowest power of compound microscope. The outer lighter-colored layer of the sponge is now seen to be made up of innumerable, needle-like *spicules*, while the inner portion is supported by radiate spicules.

Make enlarged drawings of the parts thus far considered.

Cut a thin transverse section of a dry sponge and note the radial canals, and the smaller and also straight incurrent canals lying parallel with them. Are the needle-like spicules arranged more abundantly over the peripheral ends of the radial canals? Note the arrangement of the triradiate spicules in the walls of the tubes. T-shaped spicules are arranged around the cloacal chamber, each sending one ray into the cavity. Recall the spicules of the Ascon. Do any of the incurrent canals pass through the wall of the sponge from the periphery to the cloacal chamber?

Make a drawing showing the arrangement of the spicules.

Examine now a specimen that has been decalcified, stained and cut into transverse sections by means of a microtome. The spicules will not be present though their surrounding *mesodermal cells* will appear as a delicate nucleated reticulum extending between the parallel tubes and into pyramidal masses at the peripheral ends of the radial canals. *Endodermal cells*, as a single layer, line the cloacal chamber and the radial canals; but while the cells of the former are flattened out and form a "pavement epithelium," those of the radial canals retain the *flagella* and *collars* present in the Ascon, and by their activity induce the circulation of sea-water. Note that the incurrent canals are lined with

pavement epithelium, *ectoderm*, quite different from the lining cells of the radial tubes.[1] The incurrent pores through which the sea-water enters the radial tubes are small and somewhat difficult to demonstrate. They may frequently be found in section, as interruptions of the even course of the endodermal lining along the peripheral portion of the radial tubes. They are more easily determined as minute openings in such portions of the section as permit the examination of the *surface* of the entoderm.

Make a drawing of the cross-section just examined.

Reproduction. — In the stained specimens there will frequently be found large amœboid cells in the substance of the mesoderm. Such cells, as was the case in the Ascon, are the reproductive cells, male or female, and in a single sponge may occur in varying stages of development. The sperm-spheres and the segmenting ova are clearly covered by a layer of epithelial cells. The sexes are usually not united in the same sponge-individual.

Draw one of the reproductive cells and show its nucleus and nucleolus. Draw also a sperm-sphere or a segmenting ovum, showing its position in the mesoderm and its epithelial covering.

[1] The difference in the lining of incurrent and radial canals is clearly seen in specimens that have been cut at right angles to the axes of these canals. While the endodermal tubes appear as circles of broad outline, the ectoderm of the incurrent canals forms a most delicate circle.

THE HYDRIFORM STAGE OF A CAMPANULARIAN HYDROID

(*Campanularia sp.*).

External Anatomy. — Examine with a low power and note the more or less branching *hydrocaulus* or *stem*. It is supported upon a root-like expansion or *hydrorhiza*. What is the order of the branching? Note any structures that may give flexibility to the horny, tube-like covering or *perisarc*. Do you find the perisarc enlarging at its free extremities into bell-shaped cups or *hydrothecæ*? The hydrothecæ give protection to the *zoöids* or *hydranths*. Observe that certain of the hydrothecæ may become considerably enlarged, forming *gonangia*. Each ripe gonangium may contain a number of *medusa buds*.

Make an enlarged drawing, naming the several parts.

Examine a single expanded hydranth under a higher power and note the position of the terminal *mouth*. The mouth is supported upon a proboscis or *manubrium*. Around the base of the manubrium are arranged the *tentacles* which, during life, are capable of considerable movement. Below the circle of tentacles, and quite enclosed by the hydrothecæ, the *body* of the hydranth is to be noted. What is the arrangement of the tentacles in a contracted zoöid or hydranth?

Use a still higher power. Do you find certain wart-like

batteries of *nematocysts* or "*lasso-cells*" at varying positions along the tentacles? In the living specimens (or in a living *Hydra*, held under a light cover-glass and treated with dilute acetic acid), note that each nematocyst has a rounded *body*, an elongated *filament*, and, at the base of the latter, a few recurved *spines*.

Make a drawing of an expanded and of a contracted hydranth.

Internal Anatomy. — If a clean, living specimen is selected there will be little difficulty, by focussing through the transparent perisarc, in making out the internal anatomy. If alcoholic specimens are used, portions of the colony should be stained, cleared, and mounted as described in the Appendix. Observe that the perisarc of the hydrocaulus contains a fleshy axis or *cœnosarc*. The cœnosarc is made up of three layers, and encloses, as a tube, the centrally lying *cœlenteric* or "*body-cavity*." Of the three layers the *ectoderm* is the most external. It is made up of transparent, nucleated cells, and is separated from the innermost and more granular *endoderm* by a very delicate, third, transparent, *supporting layer*.

If the specimen is still alive, note the circulation of the fluid in the body-cavity. How is the circulation brought about?

Examine one of the tentacles and determine the just described layers. To which layer are the nematocysts confined? The body-cavity does not extend into the tentacles. Demonstrate, if you can, the presence of the three layers in the body-wall of the hydranth. Is the cavity of the hydranth in direct communication with the cavity of the hydrocaulus?

Make a drawing of the stem, of a tentacle, and of a hydranth, illustrating the three layers.

The Digestive System. — Nutritive material entering the mouth is reduced to a semi-fluid condition, and, by the action of the endodermal cilia lining the body-cavity of the hydranth and hydrocaulus, is carried to the various portions of the colony, where it is appropriated and digested by the individual endodermal cells.

The Circulatory System is not specially differentiated from the digestive system.

The Muscular System. — At certain points in the tentacles of favorable specimens, elongated "muscle fibres" have been described. They lie on the outer surface of the supporting layer.

The Nervous System. — In the present stage of the Hydroid a nervous system has not been found.

The Reproductive System. — The fertilized ovum of a Hydrozoan ordinarily develops into a colony of zoöids. Such a colony we have just been considering. Through a process of branching, new colonies are asexually produced, and from the colonies medusa-buds are also produced. A brief search will probably reveal the presence of several gonangia or specialized hydranths. Each gonangium, somewhat larger than a hydranth, contains a fleshy central axis or *blastostyle*, which is morphologically equivalent to the body of a hydranth. The blastostyle is produced distally into a manubrium. Is there a mouth? Are there tentacles? Do you find the same layers that were present in the hydranth? Is there a central body-cavity? Along the side of the blastostyle are medusa-buds, in varying stages of development. Which are larger, the terminal or those nearer the base? How many are there?

Draw a gonangium with its contents.

THE MEDUSOID STAGE OF A CAMPANULARIAN HYDROID.

General Anatomy. — The medusa-buds were observed to be produced asexually along the sides of a specialized hydranth, the blastostyle. If some of the older buds are examined, they will be found to be shaped like an umbrella. The concavity of the umbrella is turned towards the exterior, and the margin is provided with numerous *marginal tentacles*. Stretched across the opening of the umbrella, as a perforate diaphragm, is the so-called *velum*. The presence of a velum is characteristic of the Hydromedusæ and gives to them the name *Craspedota*.

By the convex surface the medusa-buds are held to the blastostyle, through which, for a time, they receive their nourishment. When finally mature, the buds break away, and, passing through a terminal opening in the gonangium, reach the sea-water as free-swimming medusæ.[1]

If possible, examine a living medusa and note its peculiar movements. How many tentacles are there along the margin of the disc? Do you note a centrally projecting tube, the manubrium? At its free end the mouth is situated.

[1] The terms *exumbrella* and *subumbrella* designate the aboral and oral portions respectively. The attachment of the blastostyle is at the aboral or exumbrella portion, while the concavity, surrounded by the marginal tentacles, is formed by the subumbrella portion.

Can you trace an opening leading from the mouth into a central cavity? There are radiating *chymiferous tubes* leading from the base of the cavity of the manubrium to the periphery of the disc, where they open into the very delicate, circular, *circumferential canal.*[1] Is the arrangement of the chymiferous tubes and the marginal tentacles correlative?

The Sexual Reproductive Organs lie one on each radiating chymiferous tube, and appear, when viewed from below, as wart-like projections. Their contents, as *eggs* or *spermatozoa*, are dehisced into the sea-water.

The Nervous System and Sensory Organs. — A circular nervous tract consisting of a plexus of nerve cells extends entirely around the margin of the umbrella. Fibrillæ extend from the edge of this "ring" to muscle fibres and to certain *marginal sense-organs*. The latter may be *tactile*, "*auditory*" or *visual*. The tentacles are the principal tactile organs. The so-called "auditory vesicles" are probably organs of equilibration, and medusæ being thus provided are called *vesiculate*, while those having pigmented *visual spots* are called *ocellate*. The visual spots are more frequently found at the bases of the perradial tentacles.

Make drawings that will show the important points in the anatomy of the Hydromedusa.

[1] The chymiferous tubes are more frequently four in number and determine four radii (*perradii*) of the umbrella. In certain Campanularians, however, there are eight or more of these radiating tubes.

THE HYDRIFORM STAGE OF A TUBULARIAN HYDROID

(*Pennaria tiarella*).

External Anatomy. — Examine with a low power, and note how the present specimen differs from the Campanularian in its order of branching, in the disposition of its hydrorhiza, and in the termination of the branches of the hydrocaulus. No hydrothecæ are present. How do the zoöids or hydranths differ from those of the Campanularian? Specialized gonangia are not present, but medusa-buds will be found as globular swellings along the sides of certain hydranths.[1]

Make an enlarged drawing, naming the several parts.

Examine a single expanded hydranth under a higher power, and locate the manubrium, the terminal mouth, the somewhat irregularly placed "shorter tentacles," and the whorl of "longer tentacles" arranged around the base. Is the number of tentacles constant in different hydranths? Are batteries of nematocysts present?

Make an enlarged drawing of a hydranth.

Internal Anatomy. — Does the internal anatomy essentially differ from that of the Campanularian? Do you find

[1] Hydroids that are thus unprovided with hydrothecæ and gonangia are united in the group *Gymnoblastea*, while the Campanularian Hydroids (provided with hydrothecæ and gonangia) are united in the group *Calyptoblastea*.

ectodermal, endodermal, and supporting layers? Does the body-cavity extend into the tentacles?

Illustrate the internal anatomy by diagram.

The Digestive, Circulatory, Muscular, and Nervous Systems are not essentially different from the same of the Campanularian.

The Reproductive System. — As in the Campanularian, reproduction may be asexual (branching and budding) or it may be sexual.

Though, as already noted, gonangia are not produced, medusa-buds are abundantly found attached, as was the case in the previously examined specimen, to the body or manubrium of the hydranths. An examination of medusa-buds in varying stages of development will show that they arise as simple outpushings, or *evaginations*, of the body-wall. The more mature buds (about one-sixteenth of an inch in diameter) have all the essential characters of the same of the Campanularian, though the umbrella is here strongly four-ribbed and considerably elongated. The chymiferous tubes are four in number, and lead from the cavity of the manubrium. The velum has but a small opening leading into the cavity of the umbrella, and the marginal sense-organs are visual (ocellate). Either eggs or spermatozoa are developed from the wall of the manubrium and are often in such quantity as to distort the medusa. One or two of the eggs are frequently much larger than the others, and may be thrown off before the medusa-bud breaks away from the hydranth.

Make a drawing of a mature medusa-bud.

AN ACTINIAN
(*Metridium marginatum*).

External Anatomy. — Observe in the expanded animal that the body is made up of a cylindrical *column*, terminated above by a more or less flattened *disc* and resting below upon an irregularly shaped *base*. The column is pierced with small pores, *cinclides*, and is limited above by a ring-like fold, which contains a *marginal sphincter* or *ring-muscle*. The disc is capable of considerable expansion, and may present a regular number of *lobes*. It bears numerous rows of contractile *tentacles*, the older and larger of which form the innermost circles. These tentacles are simple outpushings of the substance of the disc. In the centre of the disc is the elongated *mouth* or *stomodæal opening*, the lateral lips of which often rest against each other, and may even be united, while the terminal angles are somewhat thickened and form the *siphonoglyphes*. (In certain specimens but a single siphonoglyphe is present.) The elevated area immediately surrounding the mouth is the *peristome*. The base, or area of attachment, is an adhesive organ, expanded laterally into a *limbus*, and capable of producing a considerable amount of motion. In the living animal the internal structure is often to be seen through the transparent walls.

Make a drawing of the specimen as seen from the side, and also as seen from above.

If the living animal is irritated, the tentacles are withdrawn, the disc is lowered, and by the contraction of the ring-muscle, the walls of the column are drawn together over the free end. If the contraction goes still further, numerous white, thread-like bodies, *acontia*, are seen to ooze from the cinclides.

Make a drawing of the contracted animal.

Internal Anatomy. — With a sharp knife divide the animal from disc to base, through a plane at right angles to the stomodæal opening, and then remove from one of the halves the thin tissue of the base. The mouth will be found to lead into an elongated, thick-walled, and more or less wrinkled tube, the *œsophagus*. Is its wall in any way differentiated at the siphonoglyphes? Below, the œsophagus opens directly into the *cœlenteric chamber*, a general cavity limited externally by the walls of the column and divided, or partially divided, into numerous smaller chambers by delicate, radially arranged partitions, the *mesenteries* or *septa*. Only six pair of mesenteries extend entirely across the cœlenteric chamber and unite with the œsophagus. They are called the *primary mesenteries*, and are definitely arranged. Two pair of primary mesenteries, the *directive septa*, occupy the plane of the longer axis of the mouth, and are attached to the walls of the siphonoglyphe. They divide the animal into two lateral halves. Between each pair of primary mesenteries — indeed, between any pair of mesenteries — is a limited chamber, the *intra-radial chamber*, while between two pair of mesenteries are the *inter-radial* chambers. There are six *primary inter-radial chambers*, three on either side. Between the primary septa are the *secondary* and *tertiary septa*, partially divid-

ing the primary chamber into smaller secondary and tertiary chambers.

Excretory and Respiratory System. — The waste products of digestion are ejected through the opening of the mouth. Certain *mesenterial filaments* may (?), however, perform excretory and respiratory functions. These filaments are arranged along the free edges of the mesenteries, at the upper part appearing as an undulating line, while lower down each filament is coiled into a tangle of considerable size. Lying near the base of the mesenteries, in the deeper part of the cœlenteric chamber, are greatly elongated mesenterial filaments, the coiled acontia.

The Reproductive Organs lie near the free edges of the mesenteries on each side of the mesenterial filaments. Each gland is a somewhat convoluted structure that dehisces its contents, *eggs* or *spermatozoa*, into the cœlenteric cavity. Are mesenterial filaments, acontia and sexual organs present on the primary septa?

The Muscular System. — Though muscle fibres are present in the walls of the column, around the disc and around the mouth, the most characteristic muscles are developed along the septa, where they run vertically as *longitudinal retractors*. Are they on the inter- or intra-septal wall? Does the arrangement on the primary mesenteries differ from the arrangement elsewhere?[1]

The Digestive and Circulatory System. — The sea-water freely mixes with the products of digestion, and the

[1] The tentacular retractors are arranged on the inter-septal walls of the directive septa; on the other septa they are on the intra-septal walls.

resultant nutritive fluid is carried, by the action of cilia, to all parts of cœlenteric cavity. At the upper portion of each mesentery circular openings are to be found, placing the contiguous chambers in communication, and thus allowing free circulation. These openings, the *septal stomata*, form imaginary rings around the upper end of the œsophagus, and are possibly comparable with the circumferential canal of the Hydromedusa.[1]

Make drawings that will illustrate the more important points in the internal anatomy.

[1] The *inner septal stomata* are placed just below the peristome near the œsophagus and pierce only the primary septa. The *outer septal stomata* are near the outer-wall and pierce all the septa.

THE MEDUSOID STAGE OF A SCYPHOZOAN

(*Cyanea arctica*).

External Anatomy. — In many points of its general anatomy the present form agrees with the medusa of the Campanularian Hydroid. A rounded *aboral, abactinal,* or *exumbrella* surface is distinguishable from an *oral, actinal,* or *subumbrella* surface. The former is generally convex, while the latter is more often concave. The margin is divided into eight large lobes.

The *mouth* will be found in the center of the subumbrella, but surrounded and probably hidden by a mass of delicate fringe-like tissue, which, in life, may hang from the lower surface of the animal in four pleated folds. These folds, which are known as the *oral tentacles,* are capable of considerable extension and contraction, and direct the food to the mouth.

The oral tentacles may now be cut away, leaving only their line of attachment along the margin of the oral opening or mouth. This opening is elevated upon a short *manubrium,* and has four radiating angles which determine four main radii of the animal, the so-called *perradii.* The four radii that might be drawn between the perradii are known as *interradii.* The perradii and interradii extended to the margin pass directly through eight "*marginal sense-organs,*"

tentaculocysts, or *rhopalia*. Each sense-organ is borne in a median sinus of one of the eight large lobes, — four perradial, and four interradial.

Four *perradial pillars* are now to be observed as supports of the angles of the mouth. They are arranged radially, and at their proximal ends rest against the *circular muscle* of the subumbrella. This muscle is a broad, flat band of parallel fibres extending as a circle midway between the mouth and the margin. A pair of *radial muscles* extend into each of the eight lobes from near the peripheral edge of the circular muscle. There are, then, sixteen radial muscles, — four pairs of perradial and four pairs of interradial.

Between each pair of contiguous perradial and interradial muscles (*adradially*) a collection of highly contractile *tentacles* will be noted. These tentacles take their origin from near the peripheral edge of the circular muscle, and, during life, may be extended to an enormous degree. They are richly supplied with *nematocysts* and are the more essential prehensile organs.

The *sexual glands* (*ovaries* or *testes*) are four large lobulated organs located interradially and separated from each other by the perradial pillars.

The *velum*, characteristic of the Hydromedusæ, is not generally present in the Scyphozoa, and the term Acraspeda has hence been applied to the members of this group in distinction to the term Craspedota as defined for the Campanularian medusoid.

Digestive System. — If a blunt probe is passed through the oral opening, the *œsophagus* will be found to be short, and, in cross-section, quadrangular. The openings into the four spacious interradial reproductive sacs lead from the

enlarged *gastric cavity*, the peripheral boundary of which extends as far as the circular muscle. Four clusters of tentacle-like interradial *gastral filaments*, or *phacelli*, are to be found encircling the deeper portion of the short œsophagus. They mark the dividing line between the œsophagus and the true gastric cavity. It is from the latter cavity, immediately within the phacelli, that the reproductive sacs take their origin.

In injected specimens the *chymiferous tubes* or *gastrocanals* can be followed, in their ramifications, through the substance of the disc and into the marginal lobes. Four main canals extend perradially and four interradially, finally opening into enlarged terminal cavities, located between the radial muscle bands and below and around the marginal sense-organs.

Alternating with the *perradial* and *interradial canals* are eight *adradial canals* which expand into as many terminal chambers, one above each cluster of tentacles. The central cavity of each tentacle communicates with the terminal adradial chamber.

The Nervous System. — Near the base of each marginal sense-organ ganglia have been found, though a marginal nerve-ring has not been discovered.

A CTENOPHORAN

(*Mnemiopsis leidyi*).

External Anatomy. — The elongated gelatinous body will be observed to have a broader, two-lobed *oral pole* and a narrower *aboral pole*. From the latter eight series of *combs*, *ctenophoral rows*, or *swimming plates* extend meridionally towards the broader end, the two lobes of which (*terminal lobes*) may be folded together and cover certain structures. The *mouth* is a slit-like opening which extends from lobe to lobe, its major axis thus determining the first perradius, and the plane (*sagittal* or *median plane*) passing through it and the axis of the body would divide the animal into symmetrical lateral halves.

A pair of small papilli-form organs will be noted, one on either side of the mouth. These are the *tentacular lobes*, and a plane passing through them and the axis of the body at right angles to the sagittal plane, would divide the animal into an anterior and a posterior half, the one an exact counterpart of the other. Such a plane may then be called the *transverse* or *lateral plane*. It lies in the second perradius.

The true *tentacles* are considerably reduced. Each appears as a small opaque spot, lying in a small pocket, midway between the lateral ctenophorial rows and a little aboral to the tentacular lobe.

The ctenophoral rows are of different lengths. The four longer lie near the sagittal plane and extend over the ter-

minal lobes, and may be called the *terminal plates*, while the four shorter lie nearer the transverse plane, and may be called the *lateral plates*.

As the eight ctenophoral rows approach the aboral pole, each terminal pair become united at the margin of an aboral crater-like depression, and form a single line which passes down the wall of the depression in the plane of the first perradius. The four *lateral rows*, however, preserve their individuality until they have reached the deeper portion of the depression.

At the base of the depression is a small opaque spot, the "*sensory body.*"

If each lateral ctenophoral row is followed orally, it will be found, at its lower end, to change its character somewhat abruptly. At this point, four long finger-shaped processes will be noted which extend freely between the terminal lobes. These processes are the "*auricles*," and they lie interradially. Each auricle is somewhat flattened, its two richly ciliated edges being directed one away from and one towards the terminal lobe. The cilia of the first-mentioned edge continue, orally, the line of the true ctenophoral row. This continuation extends from the free end of the auricle aborally along the edge that is turned towards the terminal lobe until it has reached a point near the "sensory body" midway between the neighboring terminal and lateral rows. At this point it unites with a much larger fringe that has extended aborally along the inner surface of each terminal lobe from near the sagittal end of the mouth. There are four of these large fringes.

Internal Anatomy. — The opening of the mouth has been already observed. It leads into an *œsophagus*, which

is laterally compressed often to such an extent that its walls are actually in contact. A double plate is thus formed which lies in the median plane (perradially). The flattened œsophagus opens near the aboral pole into a much smaller *stomach.*

The thick layer of jelly-like tissue that lies between the walls of the œsophagus and stomach and the external integument is traversed by certain *gastro-canals*, which, as diverticula, radiate from the stomach.

If a living animal has coloring matter forcibly injected into the opening of the mouth, the course of these canals will be beautifully demonstrated.

There are eight large gastro-canals that take their origin from the stomach, of which four arise laterally and in the second perradial plane and four arise interradially. A single pair of very small canals extend aborally in the first perradial plane from the stomach to the region of the "sensory body."

Of the four lateral canals, one pair, the more external (*tentacular vessels*), extend directly to the tentacles, while the other, deeper pair, run along the wall of the œsophagus as the *œsophageal* or *gastric vessels.* Each of the four interradial canals branches dichotomously soon after leaving the stomach. Eight *meridional vessels* are thus formed which lie under and parallel to each ctenophoral row.

It is on the walls of the meridional vessels that the *eggs* and *spermatozoa* are developed. These reach the sea-water after passing through the stomach and œsophagus.

THE STARFISH

(*Asterias sp.*).

External Anatomy. — Note the small, cylindrical *body*, the radiating *arms*, the centrally placed *mouth*, and the red *madreporic plate*. A vertical plane passing through the madreporic plate and thence across the body and along the median line of the opposite arm will divide the animal into a right and a left half. There is then bilateral symmetry.

Do you find specialized *spines* surrounding the mouth? How many? Are the spines specially developed on other portions of the animal? Note the *ambulacral furrows*, grooves, which extend along the *oral* surface of each arm. Each groove is filled with two zigzag rows of *ambulacral suckers*. Do you find any structure on the *aboral* side of the arms that suggests a median radial line? Find the *pedicellariæ*, minute pincer-like organs around the bases of the aboral spines. On what other portions of the animal are they present?

Draw the animal as seen from above, and again, as seen from below.

With a strong pair of scissors remove the entire aboral surface of the animal and without in any way injuring the underlying "soft-parts."

The Digestive System. — The mouth leads through a short *œsophagus* into a thin-walled *stomach*, the five lobes of which normally lie in the cavities of the five arms, though certain lobes, and indeed at times, while feeding, the entire stomach, may be completely everted through the mouth. Do you find the *gastric retractors*, bands of muscle which draw the lobes of the stomach into the cavities of the arms? The lobulated *digestive glands* appear in each ray as a pair of voluminous masses of a light olive-color. Each gland unites with its fellow, centripetally, and opens, through a common pore, into the stomach. Note the suspending *mesenteric fold.*

Inflate the stomach by blowing into the oral opening. The organ will be found to lead, above, into a *short* cone-shaped *intestine*, which opens to the exterior through a minute pore, the *anus*. Leading from the left wall of the intestine is a somewhat branched organ, the *respiratory tree*. It is made up of two main divisions, separated by an interradius. The organ may perform an excretory function.

The Reproductive System. — Though the *testes* and *ovaries* bear a superficial resemblance to each other, the sexes are separate. The *sexual glands* appear in mature specimens as a pair of light-colored, grape-like masses, extending from near the body out into each ray.[1] Trace the ducts to their external openings. (The openings may be found well into the angles between the arms.)

Draw the reproductive system.

[1] The testes are in living specimens of a whitish color, while the ovaries are pale yellow.

The Water-vascular System should be studied in a specimen that has been artificially injected. The ambulacral suckers were noted above as a series of tube-like organs lying along the ambulacral furrows. Each sucker passes upward, aborally, through a pore, the ambulacral pore, and expands within the cavity of the arm, into an *ambulacral vesicle* or *ampulla*. The ampullæ of each arm are in communication with a common *radiating water-tube*, which lies in the median line of each ray beneath the ridge of calcareous *ambulacral ossicles*. Follow the radial tube centripetally. It will be found to arise from a *circum-oral* ring. The *Polian vesicles*, ten in number, are enlarged ampullæ, arranged in a circle around the mouth. The *racemose vesicles*, nine in number, have their axes lying at right angles to the Polian vesicles and extend horizontally into the cavity surrounding the œsophagus. At the place where the tenth vesicle might occur a rigid tube, the *stone-canal*, takes its origin. The stone-canal extends to the lower side of the madreporic plate.

Make a drawing of the water-vascular system.

The Circulatory System.— A delicate sac, the "*pericardium*," will be found lying immediately posterior to and beneath the stone-canal. Careful injecting will show that from this sac certain minute blood-vessels pass to varying parts of the body, following, in the main, the course already taken by the water-vascular system.

The Nervous System. — Part the ambulacral suckers from the median line of each furrow, and note the deeply lying nerve-cord, the "*radiating nerve*." It extends from a *circum-oral nerve-ring* to a red *eye-spot*. The latter occupies a position at the tip of the arm.

Draw the nervous system.

With a strong knife, make a cross-section of one of the arms. Note the *perivisceral cavity* and identify the contained organs. Observe the arch of the aboral integument, and below, the large furrowed *ambulacral ossicles.* Where the ambulacral ossicle of the right meets that of the left, do you find the cross-section of the radiating ambulacral water-tube?

Remove the aboral integument from one of the arms and note that the ambulacral plates form an unbroken *ridge.* Trace this ridge to the tip of the arm and draw it, somewhat enlarged.

Remove the aboral integument from the body, taking care not to disturb the madreporic plate. Clean the skeleton and note any local specializations of the ambulacral plates. Do you find *inter-radial partitions?*

Draw the skeleton of the body.

AN ECHINOID

(*Arbacia punctulata*).

External Anatomy. — Compare the oral and aboral surfaces with the same of the starfish. Note that there are five *ambulacral areas*, separated by five *inter-ambulacral areas*. Both areas bear numerous regularly arranged *spines*. Note the "ball and socket" joint that permits movement of the spines. Are all the spines, of different parts of the body, of the same general structure? Where are they most elongated?

The aboral region is centrally free from spines, and is called the *periproct*, in contradistinction from the membranous tract around the mouth, the *peristome*. Find ten *large ambulacral suckers* on the peristome. Are *pedicellariæ* to be found on the peristome? On other portions of the body? How do they differ from the same organs of the starfish? The periproct will be found to be perforated at its centre by the opening of the *anus* which is covered by four triangular valves.

Forming the periphery of the periproct, are five radiating plates, the *genital plates*. Each genital plate will be found to form the apex of the *inter-ambulacral series*, and to be perforated by a small opening leading from the sexual glands. One of the genital plates will be found to be larger than its fellows and to appear more or less granular. It is the

madreporic plate, and is directly comparable with the same of the starfish. Arranged between the genital plates, and consequently at the apex of the ambulacral areas, are the five small *ocular plates*.

Make drawings of the animal as seen from above and from below, naming the parts; also make enlarged drawings of a spine with its "ball and socket" joint and of a pedicellaria.

Remove the spines and thoroughly wash the shell. The ambulacral and inter-ambulacral areas will be clearly shown. Find the openings through which the ambulacral suckers may be thrust out. What is the arrangement of these openings, the *ambulacral pores?*

Make a drawing of the denuded shell.

The Digestive System. — Around the margin of the mouth the five hard *teeth* will be observed. With a pair of scissors carefully remove the membranous peristome and wash out the body-cavity with clean water. The *œsophagus*, after passing through the complicated dentary apparatus, inclines to the wall of the shell, where it passes into the somewhat enlarged and elongated *stomach*. Breaking the shell if necessary, follow the course of the alimentary tract to the anus, noting any *mesentery* that may hold it in place, and observing the structure of its free border.

By a diagram illustrate the course of the alimentary tract.

The Reproductive System. — The sexes are separate, though, as in the starfish, the sexual glands superficially resemble each other. The glands appear as five radially arranged masses, and are closely attached to the *perivisceral walls*. Each gland opens to the exterior through the pore already noted in the genital plate.

Draw the reproductive system.

The Water-vascular System. — A tube, the *stone-canal*, leads from the *madreporic plate* to a *circum-oral canal* surrounding the œsophagus. From the circum-oral canal a *radiating tube* passes along each ambulacral tract, giving off branches to the several *ampullæ*. The ampullæ in turn supply the *suckers*. A so-called *heart* is to be found near the upper end of the stone-canal.

The Nervous System. — A delicate nerve forming a *circum-oral commissure* surrounds the œsophagus inside the dentary apparatus. Nerves extend from it radially, along the inner surface of each ambulacral series, to the terminal ocular plates, where they end in the eye-spots.

The Muscular System. — A number of minute muscle fibres will be found at the base of each spine, and a more or less complicated series is associated with the dentary apparatus. Find the *protractors* and *retractors* of the dentary apparatus. The *inter-alveolar muscles* are arranged between the alveolar plates, which latter hold the teeth.

Make drawings illustrating the arrangement of the muscles.

A HOLOTHURIAN

(*Thyone briareus*).

NOTE the general shape of the *body*, the point of origin of the *tentacles*, their number, and the *oral* and *anal* openings. The body is covered with papilliform *ambulacral suckers*, which, by careful observation, will be found to be arranged in five broad, meridionally disposed bands. On the upper side of the animal the suckers are less abundant than on the lower side. In the angle between the upper tentacles, the opening from the genital duct will be found. Examine the oral opening. Are there teeth? Are the ten large circum-oral ambulacral suckers of the sea-urchin represented?

Make a drawing of the animal as seen from the side.

With a pair of scissors open the animal longitudinally along the middle of the lower surface. Why is it not strictly correct to here speak of the "ventral surface"?

The Digestive System is greatly elongated and extends as a coiled tube which partly fills the body-cavity. It is poorly suspended to the body-walls by a delicate "mesentery," and in certain portions of its course becomes somewhat glandular. The mouth opens into an *œsophagus* which is surrounded by a cartilaginous structure that recalls the dentary apparatus of the urchin. The œsophagus soon enlarges into

a thin-walled *crop* or *stomach*, from which the coiled *intestine* takes its origin, and continues to its posterior opening into the *cloaca*. After making a careful drawing, remove the alimentary tract, cutting it just back of the stomach and anterior to the cloaca.

The Respiratory and Excretory Systems. — Lying laterally in the body-cavity are the two main divisions of the many-branched *respiratory tree*. Each half of this organ will be found to open directly into the cloaca, through which it may receive fresh sea-water and become greatly distended.

Make a drawing of the respiratory tree and the cloaca.

The Reproductive System. — The *ovaries* and *testes* superficially resemble each other. The sexes are separate. The *genital gland* (ovary or testis) occupies a median position in the upper part of the body-cavity, and is made up of a multitude of delicate filaments which collectively form a brush. The organ is divided into a right and into a left half, and its duct leads directly to the opening already noted, between the two uppermost tentacles.

Make a drawing of the reproductive system.

The Water-vascular System. — In certain specimens the circum-oral *water-tube* or *ring-canal* will be found to surround the deeper portion of the œsophagus, and to give off one or two large and elongated *Polian vesicles*. Communicating with the tentacles, the ring-canal gives them their power of expansion, and it also gives off five *radiating canals*, which extend over the body and supply the innumerable ambulacral suckers. Elongated filiform *ampullæ* are abundant and hang from the inner surface of the body-wall. The

madreporic plate and *stone-canal* are much reduced. The former will be found, as a small calcareous sphere, in the forward part of the body. It communicates with the ring-canal by a slender tube (*stone-canal*), lying below and parallel to the genital duct.

Make a drawing of the water-vascular system, so far as you have been able to determine its course.

Muscular System. — Note five *meridional bands* of muscles extending the entire length of the animal. Is the median band above or below? How are the *retractors of the tentacles* and of the *œsophagus* arranged? What is the arrangement around the cloaca? The body-wall is toughened by the presence of many *circular muscle fibres*. How are they arranged?

Make a drawing that will illustrate the muscular arrangement of the fore and of the hind end of the body.

The Nervous System is not easily dissected. There is a *circum-oral nerve-ring*, from which extend five *radial divisions*.

A TURBELLARIAN WORM

(*Bdelloura candida* or *Bdelloura propinqua*).

Two species of *Bdelloura* are abundantly found attached to the under surface and in the "gill-books" of Limulus. The larger species (*candida*) may reach a length of 15 mm., while the smaller, often found aggregated in clusters on the cephalothoracic appendages, does not exceed a length of 8 mm. Certain internal characters also separate the species.

External Anatomy. — The body is broad and flat, the anterior end elongated and pointed, the posterior wide and provided with a ventral *sucker*. The *eyes* are deeply pigmented, and the brownish *intestine* often gives a darker color to the middle region of the body. On the lower side the *proboscis* hangs freely from near the middle of the animal, bearing at its extremity the *mouth*. In the living animal active cilia will be found especially abundant along the margin of the body.

The Digestive System. — The opening of the mouth leads into the *pharyngeal cavity*, contained in the proboscis, and from the proximal portion of the cavity the branching intestine takes its origin.

The intestine should be examined in a specimen that has been compressed under a heavy cover-glass. It will

appear as a greatly lobulated organ, extending nearly from end to end of the animal, its lateral subdivisions giving a segmented appearance. It is built on the Tricladid type, one main branch or *ramus* extending anteriorly, and two extending posteriorly. Both anterior and posterior rami give off lateral lobes, the *diverticula* above mentioned, the rounded ends of which frequently becoming further subdivided.

The Nervous System. — The pigmented eyes are surrounded in the living animal by a halo-like area, which marks the location of the *brain*. This organ consists of a pair of lobes, united across the median line by a *commissure*. From the brain certain *sensory nerves* extend anteriorly, as transparent lines, while extending posteriorly, as transparent bands, are the *longitudinal nerve trunks*, one on either side. Note that each longitudinal nerve trunk, after extending almost the full length of the animal, is united to its fellow of the opposite side by a broad *posterior commissure*. Other commissures will be found in certain favorable specimens to pass transversely across the animal, and to extend laterally beyond the longitudinal trunks, nearly to the margins. These are the *transverse commissures*, and their number is about the same as the number of pairs of intestinal diverticula.

The Reproductive System. — The Turbellaria are mostly hemaphrodite. In the present species the *ovaries* are to be found in adult animals as a pair of rounded organs, located in the anterior portion of the body between the second and third or third and fourth intestinal diverticula. They are often more readily found if the mirror of the microscope is shielded by the hand, thus viewing the

animal with reflected light. Frequently the *oviducts* may be traced as a pair of straight lines leading directly backwards from the ovaries. The *testes* appear as rounded bodies, abundantly clustered along the margin near the ends of the intestinal diverticula, and in *Bdelloura propinqua* extending inwards between these lobes. The *seminal ducts* lead finally into elongated *seminal vesicles*, which extend posteriorly parallel to the sides of the pharynx, and finally curve towards the median line. They are quite easily found, being of an opaque gray color. The seminal vesicles, often filled with spermatozoa, open into a median pocket, the *atrium*, which in turn opens to the exterior from the oral or ventral side of the animal through a single pore. The *ova* are conveyed to this atrium through the oviducts, which are often difficult to follow. Lying lateral to the seminal vesicles, one on either side, are certain disc-shaped organs, somewhat larger than the ovaries, though often less distinct. The function of these organs is at present unknown.

The Muscular System. — Both species of *Bdelloura* are active swimmers, and the muscular system is especially developed, though too difficult of demonstration to be now considered.

Drawings should be made of the several systems.

A POLYCHÆTOUS ANNELID

(*Nereis virens*).

External Anatomy. — The elongated body is terminated anteriorly by a triangular piece, the *prostomium*, which bears certain *sense-organs*, and forms the most anterior part of the "*head*," while the posterior end is terminated by a pair of flexible *caudal cirri*. Of how many *segments* or *metameres* does the body consist? What is the arrangement of the lateral appendages? Note, through the transparent skin, the *median dorsal blood-vessel*, and on the lower side the *median ventral nerve-chain*. The latter appears as a whitish line, extending almost the entire length of the animal, and presents enlargements, *ganglia*, for each body-segment.

The prostomium is borne on an enlarged ring, the *peristomium* or *buccal somite*. The *tentacles* are small and fusiform; and take their origin near the terminal point of the prostomium, from which they abruptly diverge. Immediately lateral to and below the tentacles are the much larger and clearly segmented *palps*. In the furrow between the pro- and peristomium the *cirri*, four on each side, take their origin. Are they segmented? The *eyes* or *ocelli* are somewhat difficult to find in alcoholic specimens. They are four in number, and are arranged in pairs on the dorsal surface of the prostomium.

The *mouth* will be found as a transverse fold of the integument of the antero-ventral surface of the peristomium.

Make a drawing of the dorsal and of the ventral side of peristomium.

Observe the varying size of the lateral appendages, especially at the two ends of the animal. Carefully remove one of the larger bundles of lateral appendages and examine under a simple microscope. The bundle will divide itself into a dorsal and into a ventral *parapodium*. The former bears a large triangular blade, the *gill*. From the upper edge of the gill projects a small movable *cirrus*. Do you find ramifying blood-vessels in the gill? Do you find two fleshy *lobes* immediately beneath the gill? The dorsal lobe bears a large number of *setæ*.

The ventral parapodium is divided in much the same way as the dorsal. Do you find a ventral cirrus? Ventral setæ? Closer examination will reveal the presence in each parapodium of a supporting chitinous rod, *aciculum*.

Draw, naming the parts.

Examine the posterior end of the animal and note how the lateral appendages become reduced. Which elements are first lost? What is the point of attachment of the terminal filaments? Does the *anus* open dorsally or ventrally?

The *external openings of the segmental organs* will be found on the ventral side of each body-segment, near the bases of the parapodia.[1]

In a specimen that has been killed with extended proboscis note the large, laterally opening *jaws*.

[1] These openings are often difficult to find. They are placed at the summit of the rounded elevation that lies just medially of each ventral cirrus. The segmental organs themselves are often to be seen through the transparent integument of smaller specimens. They are opaque white in color, and lie just lateral to the longitudinal muscle bands.

Draw.

Open the animal along the mid-dorsal line, and, after gently pinning the cut edges apart, note the regular segmentation of the body-cavity. The transverse partitions or *dissepiments* correspond in position to the constrictions between the successive rings or metameres.

The Digestive System. — Cutting through the dissepiments, partially reflex the body-walls from the alimentary canal. The mouth will be found to lead into a protrusible *pharynx* which, in turn, leads into a somewhat smaller and less muscular *crop*. The crop, separated from the pharynx by a marked constriction, is in turn separated in the same way from the elongated and thin-walled *stomach-intestine*. The latter extends the remaining length of the animal. Do you find muscles connecting the pharynx to the inner walls of the peristomium?

These muscles are the "*protractors*." Their points of origin and insertion should be clearly established. The *lesser protractors* are also longitudinally arranged, but are not attached otherwise than to the walls of the pharynx. The *retractors*, unless the pharynx is extended, will be observed as fine fibres in the region of the first and second metameres. Determine their distribution.

The Crop. — Are there muscle fibres extending from the crop to the body-walls? Do you find a *gland* on each side of the crop? Trace its *duct*. How do the walls of the crop compare with those of the pharynx?

The Stomach-Intestine. — Is this portion of the alimentary tract regularly constricted throughout its entire

length? Do the constrictions invariably correspond to the surrounding body-rings? Are there any local changes in the texture or color of the stomach-intestine that might argue changes in function? Do you find a *ventral mesentery* attaching the tube to the mid-ventral line? (The dorsal mesentery has probably been destroyed in opening the animal.)

Make a drawing of the dorsal view of the alimentary tract, naming the parts.

With a pair of sharp scissors open pharynx, crop and intestine, along the mid-dorsal line. The interior of the pharynx will be found to present an anterior, tooth-bearing and a posterior, undifferentiated portion. Note the internal lining of the crop, and the small opening leading from it to the much thinner-walled stomach-intestine. Is the lining membrane of the latter different from that of the crop?

The Circulatory System in *Nereis* consists of a closed system of tubes in which the *blood*, holding in suspension numerous red *blood-cells*, circulates. The *median ventral blood-vessel* extends nearly the entire length of the animal. It lies ventrally to the alimentary tract and sends out lateral branches to each segment of the body. These *lateral blood-vessels* ramify through the gills and over the organs, uniting again, above, in a *median dorsal blood-vessel.* The latter rests upon the intestine. In the anterior portion of the animal, due to the presence of the complicated pharyngeal apparatus, the circulatory system is somewhat less regularly arranged. — The dorsal vessel is contractile, and forces the blood from the posterior towards the head.

The Muscular System. — How many distinct bands of *muscle bundles* are there? Their location? Are they segmented? How are the muscles arranged around the acicula?

Make a drawing of the muscle bands of six segments, and give special attention to the acicula and their muscles.

The Reproductive System. — The sexes are separate, and the *reproductive glands* periodically develop from the endothelium of the body-cavity. During the breeding season the *eggs*, or *spermatozoa*, often fill, as a granular or creamy mass, many of the chambers of the body-cavity. The eggs are fertilized after extrusion.

The Excretory System. — The *external openings of the segmental organs* or *nephridia* have already been noted. By gently displacing the lateral edge of a ventral muscle band, a series of small flask-shaped bodies will be exposed, lying somewhat below the ventral acicula. They are the nephridia. Trace the nephridia anteriorly and posteriorly, and see if they are present in each segment.

Make an enlarged drawing of one of the segmental organs.

Nervous System. — Find the ventral chain of ganglia. Do the ganglia of the several segments occupy the same relative position? Can you find nerves leading from them to the organs or to the body-walls? Is there anything to show that the ventral chain was originally double? Immediately back of the œsophagus, note the enlarged *infra-œsophageal ganglion*. Extending laterally and anteriorly from it are the *œsophageal commissures* which unite above the œsophagus in the *brain*, or *supra-œsophageal ganglion*. Do you find nerves passing from the brain, or commissures, to the eyes, tentacles, or palps?

Make an enlarged drawing of the nervous system.

A POLYZOAN

(*Bugula sp.*).

AFTER a colony of this Polyzoan has remained in a solution of caustic potash for a little time, all fleshy matter will be removed, and the skeleton may be washed in water and examined with a low power of the compound microscope.

The skeleton is somewhat hardened by a deposit of lime, and is known as the *cœnœcium*. It is made up of a multitude of little cups, *zoœcia*, which are arranged in double rows, back to back, each zoœcium having its opening or "mouth" directed distally. The margin of the mouth is provided with one or two spines, and on one side a deep indentation or *sinus* will be found. This sinus is on the upper surface of the double row. For convenience, the side of the zoœcium that is directed towards the median line of each double row may be called the *inner*, the side directly opposite (lateral), the *outer*, the side marked by the deep sinus, the *upper*, and the side opposite the sinus, the *lower*. The end of the zoœcium which bears the "mouth" is the *distal*, and is directed towards the free growing point; that directed towards the attachment of the double row is the *proximal*. It will be noted that the branching of the cœnœcium is always dichotomous.

On the outer margin of the sinus of the older zoœcia,

"bird's-head" structures, or *avicularia*, will be found.[1] Near the proximal ends of certain older zoœcia, on the upper surface, spherical bodies will be found. They are *egg-capsules*, or *oœcia*, and have a diameter about equal to that of the zoœcia.

By focussing deeply into the distal portion of one of the zoœcia, an irregular oval opening will be observed to lead from its inner side. This opening is the *rosette* or *communication plate* and leads into the proximal end of the zoœcium next above and on the opposite side of the double row.

In an alcoholic specimen that has been properly killed and preserved, the walls of each zoœcium will be found to extend distally and to be terminated by a circle of fourteen long *tentacles*, the zoœcium together with the fleshy parts being known as a *polypide*. The knob-shaped body bearing the tentacles is the *lophophore*. It is pierced by the mouth opening, which is placed in the centre of the tentacular crown. In life, the cilia covering the tentacles whip the food into the mouth, the pharyngeal cilia there carrying it still further into the digestive tube. The *anal opening* is to be found on the inner side of the lophophore just below the circle of tentacles. It is often more readily found if the zoœcium be examined from the lower side. Make a drawing of a polypide.

Digestive System. — The digestive tract is V-shaped. The pharynx leads into a *stomach*, which is provided with a

[1] The jaws of the avicularia, during life, are frequently closed upon small animals, the decaying bodies of which may attract smaller organisms upon which the Bugula feeds.

proximally directed pouch, the *cœcum*. The cœcum forms the lower curve of the V. The *intestine*, richly ciliated, extends from the cœcum to the *rectum;* the latter is often better seen on the lower side of the zoœcium. A loose mass of non-muscular tissue, the *funiculus*, extends from the lower end of the cœcum to the deeper portion (proximal) of the zoœcium. Make a diagram of the digestive tract.

The Circulatory System. — A vascular circulatory system is not present in Polyzoa, though the fluid contained in the body-cavity bears numerous cellular corpuscles. It probably becomes purified in the thin-walled, hollow tentacles.

The Muscular System. — During life the lophophore, together with the tentacles, is often forcibly retracted into the cavity of the zoœcium. This contraction is brought about by the action of certain *retractor muscles*, which may be seen in favorable specimens extending across the body or cœlomic cavity from the inner surface of the zoœcium to the inner walls of the pharynx and lophophore.

The Nervous System is represented by a *supra-œsophageal ganglion*, situated between the mouth and the anus. From it certain nerves go to the tentacles. There are no differentiated *special sense-organs*, and the entire system is too difficult of demonstration to be considered in the present course.

The Reproductive System. — The increase in the number of polypides in the colony, or *zoarium*, is brought about *asexually* by "budding," though the more mature polypides

are provided with true sexual glands. The *ova* are often found in varying stages of growth lying in the body-cavity near the cœcum. They are yellowish in color and oval in outline. The *spermatozoa* lie deeper, near the proximal end of the funiculus and form a cloudy mass.

A GEPHYREAN WORM

(*Phascolosoma gouldii*).

External Anatomy. — The elongated body shows no external traces of segmentation, and lateral appendages are not present. The anterior end is often deeply withdrawn, though in specimens killed with considerable care the *mouth* will be found to occupy a terminal position, and to be surrounded by a crown of tentacles. The *anal opening* is not at the posterior end, but at the apex of a median papilla, situated near the middle of the animal, and marking the dorsal surface.

Make a drawing of the animal as seen from the side.

Internal Anatomy. — With a pair of scissors open the specimen along the median line a little to one side of the anus and pin the body-walls so as to expose the internal organs. Note the *dorsal* and the *ventral pharyngeal retractors*. They are large muscle bundles, longitudinally arranged, and retract the anterior portion of the animal.

The Digestive System. — Locate the *mouth* and follow the coiled *stomach-intestine* to the "posterior" end of the body, thence back to its final opening on the dorsum. Do you find *mesenteries?* Are there *dissepiments?*

The Circulatory System is with difficulty followed out. There is a *dorsal* and a *ventral vessel*, much as in *Nereis*.

The Muscular System. — What is the arrangement of the muscle fibres of the integument? How many longitudinal bands are there? Examine again the pharyngeal retractors.

Draw.

The Reproductive System. — The sexes are separate. The *sexual glands* are situated near the base of the ventral pharyngeal retractors. The *eggs* or *spermatozoa* reach the exterior through the single pair of *nephridia*. Fertilization occurs after extrusion.

The Excretory System. — The *nephridia* are a pair of large brown tubes which lie, somewhat freely, in the neighborhood of the retractors. Their openings to the exterior lie a little in front of the origin of the dorsal retractors.

Draw.

The Nervous System. — The *ventral cord* lies along the mid-ventral line. Examine with a lens and see if it is provided with *ganglia*. Do nerves leave the ventral cord? Find the *œsophageal commissures* and trace them to the *brain* or *supra-œsophageal ganglion*. Are there pigmented *eye-spots?*

Draw.

A LAMELLIBRANCH

(*Venus mercenaria*).

External Anatomy. — The *shell* is composed of two bilateral halves or *valves;* the *hinge,* or their common line of union, marking the dorsal side. The anterior end is less elongated than the posterior. The *umbo* is the dome-shaped elevation along the dorsal edge of each valve. Lying dorsally, between the valves, and extending posteriorly, is the *ligament.* Which is the right valve, and which the left? Note the parallel *lines of growth.*

Draw.

Pry the shells apart and note the thickened *mantle-edge,* running parallel to the free margin of the shell. It is partly divided into a right and a left half, by longitudinal openings. The anterior and ventral opening (*pedal*) gives opportunity for the protrusion of the foot while the posterior or siphonal permits the ingress and egress of salt water.

Internal Anatomy. — Remove the left valve of the shell, taking care not to injure any of the underlying soft parts. If the animal has been killed by being allowed to stand in warm fresh water for a little time, the valves will naturally gape apart, and the attachments of the strong *adductor muscles* may be pressed from the shell with the handle of

the scalpel. Note the left *mantle-lobe* covering the whole surface of the animal, except the muscles. Through the semi-transparent mantle, the outlines of the underlying viscera may be seen.

Along the dorsal edge, midway between the muscles, portions of the dark green *liver*, the cream-colored *reproductive gland*, and the brownish *Keber's organ* are to be noted, in the order named. Further posteriorly and dorsally, between Keber's organ and the posterior adductor muscle, the *heart* may be observed, lying in the delicate *pericardial sac*. If the animal is still alive, the pulsations of the organ may be noted. Immediately in front of the posterior adductor, and lying somewhat lateral to the heart, the yellowish *organ of Bojanus* is to be noted.

The *foot* is a plough-shaped organ, pointed at its anterior end, and supported from the lower margin of the great, swollen, soft-bodied *visceral mass*.

With a pair of scissors remove the left lobe of the mantle, cutting around the anterior and posterior adductors. The visceral mass nearly fills up the cavity between the two mantle-folds (*mantle* or *pallial chamber*) and is crossed on each side, diagonally, by two fringe-like *gills*. If the gills are followed anteriorly, they will be found to end near two small gill-like organs which lie just posterior to the anterior adductor. These organs, of which there are two on each side, are the *palps*. They lie, one pair above, and one pair below, the *mouth-opening*.

Examine the *siphonal region*. The mantle will here be found to be specialized in such a way as to form two protrusible tubes, of which the lower leads from without into the mantle chamber.

Make a drawing of the above-mentioned organs.

The Muscular System. — The strong anterior and posterior adductor muscles have already been noted. Is there any difference in their relative size? Can you discover the *retractors of the foot*, smaller muscles lying near the adductors? The *siphonal muscles* lie around the base of the siphon. Note any lines of attachment on the inner surface of the removed valve that correspond to the muscles just mentioned.

The Respiratory System. — Pass a probe into the lower siphonal canal (the *incurrent canal*) and note that the canal may convey water into the mantle chamber. Into this chamber, which is also known as the *infra-branchial chamber*, the gills or *branchiæ* hang. Each gill is made up of two parallel plates of *rods* or *bars*. Study carefully the attachment of the two pairs of plates of each side to the mantle, without, and to the body-wall, within.

Inject water into the upper siphonal opening and notice that it fills a certain chamber lying above each pair of gill-plates or lamellæ. This chamber is the *supra-branchial chamber*, and on each side, above the outer and inner gills, has an *outer* and an *inner* division. The outer subdivision may be exposed by cutting along the line of attachment of the outer lamella to the mantle. The inner sub-chamber may be exposed by turning back both gills and cutting along the line of attachment of the inner lamella of the inner gill with the body-wall.[1] Look into these chambers and note the numerous openings of the *water-pores*, tubes which lead from between the gill-lamellæ.

Carefully slit open a gill in such a way as to separate its lamellæ and, floating one of the lamellæ on a slide, examine with a compound microscope. If the gill is still alive, a

[1] The inner lamella is only partially united to the body-wall.

most beautiful ciliary motion will be observed. Note the supporting bars and the openings through the gill-lamella for the passage of water. This passes from the infra-branchial chamber into the water-tubes between the lamellæ, and thence into the supra-branchial chamber. The supra-branchial chambers of the right and of the left side open into a common *cloaca*, below and behind the posterior adductor. From the cloaca the water reaches the exterior through the upper siphonal or *excurrent* canal.

Make drawings of all the parts of the respiratory apparatus. The arrangement of the supra-branchial chambers may be well shown by imaginary cross-sections.

The Circulatory System. — The location of the heart within the delicate *pericardium* has already been noted. The organ will be found to consist of a median and strong-walled *ventricle* into which opens, from each side, through an *auriculo-ventricular opening*, a thin, triangular-shaped *auricle*. The lower side of the auricle, corresponding to the base of the triangle, lies above the supra-branchial chamber and receives the purified blood from the gills. The *rectum*, or posterior portion of the intestine, passes through the ventricle. Injected specimens will clearly show the course of the arteries as they pass anteriorly and posteriorly from the heart. The *bulbus arteriosus* will be noted on the posterior aorta as a large contractile sac.

The Excretory System. — The *kidney*, or organ of Bojanus, has already been noted. Examine under the organ and near its anterior end. Two small openings will be found. The more posterior is the opening of the *ureter*, and leads from the kidney. It lies inside[1] the second, the *reproductive opening*. If a hair is carefully passed into the open-

[1] Nearer the median plane.

ing of the ureter, and the outer wall of the kidney is then dissected away, the probe will be found to have reached what is called the "*upper limb*" of the kidney. This cavity is a comparatively smooth and thin-walled sac. It is continuous with the "*lower limb*," which lies posteriorly, and is provided with more glandular walls. This *lower limb* finally communicates, through a funnel-shaped opening, with the cavity of the pericardium. The opening into the pericardial space is small and somewhat difficult to demonstrate. These renal organs are comparable to the nephridia of *Nereis*.

Make a careful drawing of the parts just studied.

Reproductive System. — The *reproductive gland* (*testis* or *ovary*) is a somewhat delicate and voluminous organ, partially enfolding the coiled intestine. The opening of its duct has already been noted.

The Nervous System. — The *left cerebral ganglion* will be found as a small body, about the size of a pin's head, lying just behind the upper portion of the anterior adductor. Find the short, transverse commissure that connects it with its fellow of the opposite side. The *visceral ganglia* lie on the lower surface of the posterior adductor. They are yellowish masses, from which numerous nerves radiate. A larger nerve may be followed from the left visceral to the left cerebral ganglion. It is the *cerebro-visceral connective*, and lies for the most of its extent near the surface. The *pedal ganglia* lie side by side in the substance of the anterior portion of the foot, and just below the visceral mass. *Cerebro-pedal connectives* may be traced through the visceral mass from the pedal to the cerebral ganglia.

Make a drawing of the nervous system.

The Digestive System. — The *mouth*, already noted, is provided with an *upper* and an *under lip*, two transverse folds that apparently connect the upper and the under palps. (The position of the mouth can be very clearly seen if the clam is carefully removed from its shell and pinned out under water.) The posterior portion of the alimentary tract passing through the ventricle has already been noted as the *rectum*. Follow the rectum in its further course over the posterior adductor to its final opening into the cloaca.

To now trace out the course of the entire digestive tube considerable care must be exercised. — Carefully pick or cut away, piece by piece, the side of the visceral mass. The short *œsophagus* will be found to lead upward and backward into the *stomach*. The latter is an irregular sac situated dorsally and anteriorly, and has, opening into it, the ducts from the *digestive gland* or *liver*. The liver almost entirely surrounds the stomach.

The *intestine* is long and coiled. It begins at the posterior end of the stomach, passes to the lower posterior portion of the visceral mass, and then curves to the left and forward, soon to again bed itself deeply into the surrounding viscera and take on several convolutions. Finally the intestine passes upward, posterior to and nearly parallel with the first section until, at the level of the stomach, it bends sharply backward to pass through the ventricle of the heart. During a portion of its course the lumen of the intestine is partially filled by a fold, the *typhlosole*. The tract does not lie in a "body-cavity."

During the dissection of the digestive tract a long gelatinous cord has doubtless been noted. This is the *crystalline style*. Its function is not known.

Make a drawing of the alimentary tract.

A CEPHALOPHOROUS MOLLUSK

(*Sycotypus canaliculatus*).

External Anatomy. — Examine an animal enclosed within its shell and note the following points: —

The *shell*, unlike that of the Lamellibranch, consists of but a single piece (*Univalve*). It is twisted towards the right, around a central axis, the *columella*, and makes several revolutions or *whorls* from its *apex* or *spire*, to its lower opening or "*mouth*." The side of the mouth nearer the columella is called the *internal lip*, while the outer, thin, convex edge is the *outer lip*. Extending from the mouth downwards (away from the spire) is an elongated groove, the *canal*. The line of union between two whorls is called the *suture*. *Lines of growth* extend as parallel series, at right angles to the suture, and parallel to the edge of the outer lip. Frequently the shell is covered externally by a felt-like horny *epidermis*.

Draw the shell from the side, naming the parts.

From the "mouth" of the shell the soft parts of the snail may be protruded, the large *foot* being the last to disappear during contraction and the first to leave the shell as the animal again expands. To the upper side of the foot a horny lid, or *operculum*, is attached, which may effectually close the opening of the mouth.

As the animal crawls about, the shell is carried upon its

back, the canal being directed forward, the spire backward. The opening of the shell is then on the right side.

Examine an animal that has been removed from the shell, and compare the number of whorls made by the body with the same of the shell.

The lower surface of the foot is somewhat wrinkled. It has, leading from its centre, an opening from the "*pedal gland.*" At the posterior and dorsal part of the foot, observe the attachment of the operculum. Extending anteriorly and dorsally, the substance of the foot merges into the *head.* The head is anteriorly provided with two laterally projecting and triangular flaps, the *tentacles.*[1] Midway between the tentacles, but somewhat below them, is the opening of the *mouth.* Frequently the elongated *proboscis* projects from the mouth as a flexible tube, an inch or more in length. If the specimen being examined is a male, the large intromittent organ, or *penis,* will be observed. It is situated a little to the right of and above the right tentacle.

The *collar* is a circular fold of the mantle that entirely surrounds the body just posterior to the head. In life, it was applied to the lips of the shell, and by its activity the body of the shell (middle layer as well as the epidermis) was elaborated. At a point corresponding to the canal the inner part of the collar is produced into a groove-like *siphon.*

If the collar is elevated along its antero-dorsal portion, a large chamber, the *mantle cavity,* will be exposed.

The soft parts of the animal, extending into the spire, are collectively spoken of as the "*visceral mass.*" It should be borne in mind that the outer surface of each whorl corresponds to the left side of the animal, while the inner surface, next the columella, is the right side.

[1] On the lateral edge of each tentacle is the small deeply-pigmented eye.

The outlines of the following organs may be distinguished through the somewhat transparent walls.

The *liver* or *digestive gland* extends as a dark green mass, from the apex of the spire, of which it forms the larger part, to a point two revolutions lower down. It bears on its dorsal surface the lighter sexual gland, *testis* or *ovary*, the sexes in this univalve being separate.

On the external surface of the liver the *stomach* is often to be seen as a curved body somewhat less than the sexual gland in size. Extending anteriorly from the liver, and confined more to the outer portion of the whorl, is the dark-colored *renal organ* or *kidney*. The outline of this organ, when viewed from above, is triangular, the apex being directed towards the columella, while the base extends laterally. Viewed from the side, the base of the triangle is seen to rest upon the liver, posteriorly; upon the *pericardial chamber*, ventrally; and upon the roof of the mantle chamber, anteriorly. The renal organ is divided into a smaller *tubuliferous portion*, which lies against the pericardium, and a larger *acinous portion*, which lies above and around the first.[1] The line separating the two runs parallel with the upper edge of the pericardium. The *pericardial chamber* is somewhat crescentic in outline, and is about the size of one of the tentacles.

If the specimen being examined is a female, the roof of the mantle chamber presents on its dorsal surface a long, large elevation of light cream-color, extending from the liver, and internal to the kidney, nearly to the free edge of the collar. This elevation is the *nidamental gland*. Through its activity the long *egg-strings* are manufactured. Lying parallel to, but outside of and below the nidamental gland (nearer to the siphon tube), the location of the gill is clearly

[1] The function of the tubuliferous portion is uncertain. It is probably not excretory.

seen. It is a large crescentic area, often slightly depressed. On the inside of the visceral mass, and extending somewhat parallel to the nidamental gland, is the large *columellar muscle*, by means of which the animal retains its hold upon the columella of the shell.

Make drawings of the animal as seen from in front and as seen from the outer and inner sides.

The Anatomy of the Mantle Cavity. — With a pair of scissors slit open the roof of the mantle chamber along a median line extending parallel to the upper edge of the gill. Reflect the flaps and observe, on the inner side of the left, the extent of the single large lamellate *gill.* What is the arrangement of the *branchial lamellæ?* Immediately below the gill is a smaller organ, somewhat similar in outline and structure. It is the *osphradium* or *olfactory organ.* On the inner surface of the right flap the external opening of the *rectum* will be noted. It is at the apex of a short anteriorly directed tube.

If the specimen is a female, the *external opening of the oviduct* will be found at the apex of an elevation near, though a little lower than, the anal papilla. Pass a flexible probe (a guarded bristle) into the opening.

Males are somewhat smaller than the females, and may be readily distinguished by the intromittent organ. Leading out from the base of the *penis*, and inclining towards the right, until it finally reaches the groove made by the union of the roof of the mantle chamber to the body, is a clearly defined ridge which marks the course taken by the *vas deferens.* Trace the vas deferens to the upper portion of the mantle chamber.

In both sexes a large gland, the *hypobranchial* or *adrectal*

gland, occurs as a series of tranverse folds along the roof of the mantle chamber. In the female the gland extends between the gill and the nidamental gland.

At the very apex of the mantle chamber a slit-like opening will be found. This opening leads from the cavity of the renal organ.

Make a drawing that will illustrate the anatomy of the mantle chamber.

Internal Anatomy. — The systems of organs may be dissected in the following order: —

(*a*) **The Circulatory System.** — Though it is well to use a hypodermic syringe with some colored liquid for tracing out the arteries and veins, the larger blood-vessels may be located in the uninjected specimen. — The *branchial vein,* seen from without, is a transparent line running along the lower side of the area which we know to be that of the gill. It may be followed nearly to the pericardium, where it unites with a *renal vein* that returns the blood from the lower, tubuliferous portion of the renal organ. The common vein empties into the *auricle* of the heart.

With a pair of scissors carefully open the pericardial chamber, and gently lift the heart from the cavity. The auricle will appear as an anterior division, thin-walled and collapsed. The *ventricle,* lying posteriorly, is much larger, and is provided with strong muscular walls. From the ventricle arise two arteries. These pass from its posterior end and convey blood to all parts of the animal. The *visceral artery* passes upwards, through the substance of the liver, giving off branches to the stomach and reproductive gland. By the careful use of probes the visceral artery is not difficult to trace. The *aorta,* the second artery,

makes an abrupt turn downwards and forwards, and passes towards the head. It soon enlarges and forms a *secondary heart* which lies, as dissection will show, to the left of, upon and to the right of, the œsophagus. Large branches are given off to the muscles of the foot, siphon, and to the head.

Make a drawing of the heart and principal blood-vessels.

Cut the heart in two and reflect, exposing the pericardial chamber. Note the serous lining, and especially the opening which leads into the renal organ. This opening, which is of considerable morphological significance, will be found somewhat below and inside of the point of entrance of the branchial and the renal veins, and near the deepest point of the pericardial chamber.

Make a drawing of the interior of the pericardium.

(*b*) **The Excretory System.** — The location of the glandular and of the tubuliferous portions of the kidney have already been noted. With a small pair of scissors cut along their common line of union, opening the chamber of the kidney. Observe the numerous parallel lines of *tubules* which make up the substance of the lower, *tubuliferous portion.* The larger, *acinous portion* of the kidney is made up of large lobules over which blood-vessels ramify, on their course from the upper part of the body to the gill. Look for the large opening leading from the renal to the mantle chamber, and also for the smaller opening which leads from the pericardial chamber.

Make a drawing of the glandular organs of the kidney.

Carefully remove the acinous portion of the renal organ.

(*c*) **The Reproductive System.** — If the specimen being examined is a female, the *ovary* will be found as already noted. Trace the *oviduct* as it leaves the lower inner side

of the ovary and passes down to the nidamental gland. In the living animal it rests against the columella. Follow the course through the lumen of the nidamental gland to the exterior. Open the ovary and note the ultimate branches of the oviduct and the numerous *eggs*.

Make drawings that will illustrate the anatomy of the ovary and the course of the oviduct.

The *testis* of the male lies in the same place as does the ovary of the female, and its duct, the *vas deferens*, pursues a similar course along the inner surface of the liver to that taken by the oviduct. The course along the floor of the mantle chamber has already been noted.

Draw.

(*d*) **The Digestive System.**—Select a specimen that has been killed while the proboscis remained protruded and note the terminal *mouth-opening*, and the *lingual ribbon* or *odontophore*, projecting slightly from its lower side. With a pair of scissors slit open the proboscis along its mid-dorsal line, taking care to cut only through the skin. Immediately below the incision, the light-colored *œsophagus* will be seen as a straight tube. It extends from its opening into the *pharyngeal cavity*, immediately over the end of the odontophore, to the upper end of the proboscis, where it abruptly dips into the substance of the body. Note the small fibres that attach it to the integument, and which, at its outer end, are deeply colored, and may, by their contractility, give the organ a pumping function. Extending from the base of the proboscis into the substance of the body are a pair of white muscle bundles, the *right and left retractors of the proboscis*.

Below the œsophagus lies the deep-colored *muscular mass* that brings about the movement of the lingual ribbon.

Draw.

The *odontophoral* or *radular protractors* are muscle fibres which extend from the lower side of the muscular mass to the inner surface of the integument, near the free end of the proboscis. The *radular retractors* are a series of fibres extending dorsally from the proximal end of the cylinder to the inner surface of the integument, near the base of the proboscis.

Having opened the œsophagus, free it from the underlying muscular mass, and note the transverse fibres of a thin muscle sheet that rested below the middle third of the œsophagus and gives attachment to the radular protractors along its lateral edge. Observe that the radula extends under the muscle from near the base of the proboscis to its free end, and that the obliquely disposed radular retractors also extend under the muscle sheet.

Carefully slit open the muscle sheet and note the place of insertion of the radular retractors along the sides of the radula.

The lower surface of the radula rests upon a pair of elongated *odontophoral cartilages*, the retractor muscles of which extend proximally and below the radular retractors to a tendinous ring, which also gives attachment to the white fibres of the retractors of the proboscis. On the ventral side of the muscular mass the *ventral retractor of the radula* will be noted. It is a small muscle, lying between the odontophoral cartilages, and by its contraction the dorsal surface of the radula is brought down over the pulley-like ends of the odontophoral cartilages.

Make a drawing that will illustrate the structure of the radular apparatus.

The œsophagus passes between the mass of white re-

tractors of the proboscis and midway between the large yellow *salivary glands.* The *ducts* from the salivary glands appear as two delicate tubes extending one on either side of the œsophagus as it passes down the proboscis.

Draw.

At a short distance beyond the salivary glands the œsophagus is surrounded by the *orange-colored ganglia* of the nervous system. A little posterior to the œsophageal nerve-ring, the alimentary tube receives the duct from a delicate, elongated gland, the "*pancreas*," which lies to the right side of and upon an enlarged section of the alimentary tract, the *crop.* The crop becomes smaller in diameter as it approaches the liver, into the lower portion of which it courses, and becoming again enlarged, forms the "*stomach.*" The walls of the stomach may be seen without dissection as a whitish tract on the outer surface of the liver.

If the stomach is opened and the contents washed away, the large *hepatic ducts* will be found. How many ducts are there? After passing along the lower side of the liver, the stomach bends abruptly upward and forward, and thence, as the *intestine* and *rectum*, runs to its external opening parallel to the crop and œsophagus. Is the epithelial lining of the rectum in any way specialized?

Make drawings that will illustrate the course of the alimentary tract.

(*e*) **The Nervous System.** — The nervous system of the Cephalophorous or Gasteropod mollusks consists of a series of *ganglia*, chiefly arranged around the œsophagus, and of a number of radiating *nerve-cords.* — The circle of ganglia surround the œsophagus somewhat in front of the duct of the "pancreatic gland," and when viewed from above present

considerable bilateral asymmetry. If the anterior part of the œsophagus is turned back, however, the lower side of the ganglionic mass will be found to be made up of two nearly equal halves.

Lying ventrally are the small *buccal ganglia*, a pair of spherical bodies somewhat in front of the remaining ganglia and giving origin to a number of nerve-strands that pass to the pharyngeal apparatus. A *buccal commissure* runs beneath the œsophagus and connects the right with the left ganglion.

Also, on the ventral side of the œsophagus are the much larger *pedal ganglia*. These lie, fused together, a little posterior to the buccal ganglia, and from them large strands of nerves pass to the right and left halves of the foot.

Cerebro-pedal connectives pass from the pedal ganglia dorsally, partially encircling the œsophagus, and uniting with the *cerebral ganglia*. The cerebral ganglia rest dorso-laterally upon the œsophagus, and are united dorsally by the *cerebral commissure*. Nerves pass from the cerebral ganglia to the region of the head. *Cerebro-buccal connectives* unite the buccal and cerebral ganglia.

The left cerebral ganglion is in union ventrally and posteriorly with the *left pleural ganglion*, which rests anteriorly upon the posterior part of the left pedal ganglion.

The right cerebral ganglion at first appears to be somewhat larger than its fellow. Examination will show that this is because of its partial fusion with the *right plural ganglion*, which lies immediately ventral and posterior to it.

At the posterior edge of the right pleural ganglion, the pear-shaped *right visceral* or *supra-intestinal ganglion* is seen to be attached. It lies almost directly upon the dorsal surface of the œsophagus, and gives off several branches, of which two go to the osphradium, and a third passes pos-

teriorly in a line nearly parallel to the course taken by the digestive tract. This posterior band forms one half of the so-called *visceral loop* and leads to the *abdominal ganglion*, located far up in the substance of the body near the nephridial opening.

The *left visceral* or *sub-intestinal ganglion* lies on the right side of the œsophagus, below the right pleural ganglion, and posterior to the right pedal ganglion. A broad band, the *left viscero-pleural commissure*, will be observed to lead from the left visceral ganglion to the left pleural ganglion, passing below the œsophagus just back of the pedal ganglia. The left visceral ganglion gives rise to the left half of the visceral loop, which passes posteriorly to join the abdominal ganglion already noted. On its course it gives off a branch to the columellar muscle.

The pleural ganglia give off nerves which pass to the lateral walls of the body and to siphon and collar.

Make a series of drawings of the nervous systems.

A CEPHALOPODOUS MOLLUSK

(*Loligo pealii*).

External Anatomy. — Note the elongated body, separated from the movable *head* by a constricted *neck*. The integument is abundantly supplied with *chromatophores*. Though not of the usual morphological significance, observe that there is an anterior and a posterior end, and a dorsal and a ventral surface. What is the shape and attachment of the terminal *fins?* Demonstrate that there is an opening between the neck and the "*collar*" into a central chamber, the *mantle cavity*. What is the position of the *eyes?* Are they provided with *lids?* How many *arms* are there? Are all of the arms of the same structure? How many rows of *suckers* are there on the shorter arms? On the longer? Do you find the *mouth?* The opening of the *siphon-tube?* Just back of each eye, note the fold of the *olfactory organ*. What is its extent? Note the median, dorsal projection of the collar. Turn it back, and note that it is stiffened by the presence of a horny structure.

Open the animal along the mid-dorsal line. The horny structure, *pen*, will be found to extend the entire length. Remove the pen.

Make a drawing of the animal as seen from the left side, putting in as much detail as possible and naming the parts. Also make a drawing of the pen.

Examine one of the larger *suckers*. Is it sessile or mounted on a *peduncle?* Note the horny *ring*. Is the margin of the ring entire or serrate? Make a longitudinal section through the sucker and its fleshy *piston*.

Draw the side view of the section.

Internal Anatomy. — Carefully open a male specimen, a little to one side of the mid-ventral line, exposing the *mantle cavity*. Pass a large probe through the opening of the siphon. Do you find the large *retractor muscles* of the siphon? Trace them to their posterior points of attachment. On each side of the siphon note the lateral *siphonal blind-sacs*. What is their function? Immediately posterior to the siphon note the tube-like *rectum*.

The *anus*, at the most anterior point of the rectum, will be found guarded by two lateral, leaf-like valves. Carefully inject water into the rectum, and note the outline of the organ.

Immediately dorsal to the rectum is a dark-colored sac, the *ink-sac*. Slit open the anterior end of the rectum, along its mid-ventral line, and note the point of entrance of the tube from the ink-sac.

Lying along each side of the mantle cavity, and parallel to the retractors of the siphon, are the *gills*. How is each gill attached? Do you find, at the base of each, a disc-shaped *branchial heart?* The *median mantle artery* will be noted, as a cord-like tube, extending from a point midway between the branchial hearts, across the mantle cavity to the inner side of the mantle. It marks the anterior limit of a *median mesenteric fold* that extends from it posteriorly.

Looking through the transparent tissue that covers the viscera, a pair of underlying *urinary organs*, or *nephridia*,

will be seen. Anteriorly, each organ opens through a small pore that thus leads from the nephridium to the mantle cavity. Each pore lies near the median line, some little distance in front of the branchial heart, and marks the anterior end of the nephridial chamber. The posterior and broader end of the chamber lies just medially of the branchial heart, the right and left nearly meeting in the median line.

Two arteries, *lateral mantle arteries*, will be noticed (one on each side), extending laterally and posteriorly, from near the point where the median mantle artery leaves the visceral mass, to the internal surface of the mantle. Lying laterally to each lateral mantle artery is a much larger thin-walled *posterior vena cava*. This organ is often filled with blood that has been received from the *posterior mantle veins* which lie parallel to the mantle artery. The cone-shaped posterior portion of the mantle cavity is filled up with the voluminous *visceral sac* and the *sexual organs*.

Make an outline drawing of the organs as they naturally lie in the mantle cavity.

The Circulatory System. — By the use of a hypodermic syringe the circulatory system may be injected and the following points determined.

Each post-cava empties its blood into the branchial heart of the same side, which also receives the blood that has been returned from the anterior portion of the animal through the *precavæ*. The latter traverse the glandular walls of the nephridial chambers. Each branchial heart also receives an *anterior mantle vein*, returning blood from the anterior portion of the mantle. Impure blood is forced from the branchial heart to the gills along the *afferent branchial vessel* which traverses the edge of the gill that is directed towards the mantle.

The pre-cavæ unite just in front of the nephridial organs and form the *common vena cava.* This vessel lies anteriorly in the median line, just dorsal to the ink-sac and midway between the siphonal retractors. It returns the blood from the ink-sac, liver, œsophagus, and head.

The *systemic heart* lies in the median line between the posterior ends of the nephridial organs and receives blood from the *right* and left *efferent branchial vessels,* which course along the free edge of the gill.

The systemic heart gives off an *anterior* and a *posterior aorta.* The former passes directly to the head, dividing at the neck into a *right* and a *left branch.* On its course it gives off certain branches to the digestive organs. The *posterior aorta* soon divides into the median and lateral mantle arteries already noted.

The cavæ rest dorsally on a delicate flask-shaped bilateral organ of considerable size, the "*spleen.*"

Make drawings illustrating the course of the blood-vessels.

The Digestive System. — Note the large chitinous *jaws,* situated at the base of the circle of arms. Which jaw is larger? Open the mouth and note the *lingual ribbon* or *odontophore.* What is its position? Pass a probe down the narrow *œsophagus,* and cutting along the mid-ventral line of the head and neck, follow the œsophagus to its entrance into the *stomach.* On its way it is accompanied for a short distance, along its ventral side, by the duct from the *median salivary gland,* a small body which may be found enfolded in the anterior end of the liver. The *lateral salivary glands* lie on the posterior surface of the buccal-mass. The *liver* is a large gland that extends posteriorly from near the spherical buccal-mass to a point dorsal to the nephridial

openings. It lies between and dorsal to the siphonal retractors, and is pierced at its anterior end by the œsophagus, which courses along its dorsal surface.

The œsophagus finally enters the smaller, more muscular, *cardiac portion* of the stomach. By blowing with a blowpipe into the cardiac portion, the much larger and thin-walled *gastric sac* will become inflated. This organ is a "blind-sac" and extends to the posterior end of the mantle cavity. It often contains partially digested food — bits of fish, etc.

Trace the *intestine* from its origin, near where the œsophagus enters the stomach, to the rectum. If the gastric sac is already filled with water, it may be rolled from its original position, clearly showing its relations to the other neighboring organs.

Make a drawing of the entire digestive system as seen from below, and also as seen from the side, naming all the parts.

The Muscular System. — The Cephalopods are most active Invertebrates and the muscular system is correlatively developed. Muscle tissue largely makes up the outer substance of the animal, though there is a remarkable lack of clearly defined muscle bundles. The retractors of the head and siphon are, however, more definitely isolated.

Reproductive System of the Male. — As the gastric sac is partly removed, note on the posterior dorsal wall of the mantle cavity the large *testis*. It is firm in texture and somewhat elongate-oval in outline. Leading to the testis from the region of the cardiac stomach is an artery which pierces the delicate capsule in which the organ lies. At the left of the testis, the *accessory reproductive organs of the male* are to be observed. They form an irregular mass

from which a tube, the *spermatophoric sac*, passes anteriorly to open through the *penis*. The latter is a tube of considerable size, lying parallel to, and along the left side of, the rectum. The penis finally opens at a point about even with the anus.

There extends from the spermatophoric sac, which is often filled with numerous small worm-like *spermatophores*, to the capsule of the testis, an elongated and much convoluted tube, the *vas deferens*. It is, in places, considerably swollen and highly glandular.

Make a drawing of the male sexual organs, naming the parts.

The Reproductive System of the Female. — If the mantle cavity of a female specimen is opened in the mid-ventral line, the sex may be at once determined by the presence of two large *nidamental glands*, each about the size of one's little finger and lying side by side along the median line, well towards the anterior of the mantle cavity. Each gland has an anterior opening through which the gelatinous substance of the *egg-capsule* is extruded. Lying in front of the nidamental glands are the smaller, reniform *accessory glands*, which rest laterally upon the sides of the rectum and ink-bag.

The large funnel-shaped *opening of the oviduct* will be seen at the left, on a line with the openings from the nidamental and accessory glands. Pass a probe into the oviducal opening, and trace the duct below the left gill into the large *oviducal gland*. It is situated on the left of the posterior end of the nidamental gland. From the central point of the oviducal gland the more constricted *oviduct* will be seen. It passes posteriorly towards the mid-ventral line.

Follow the oviduct far back and note the abrupt turn in its course as it bends anteriorly. Trace it to its opening into the capsule of the ovary. The ovary lies along the dorsum and extends anteriorly from near the posterior end of the animal to a point dorsal to the external opening of the oviduct. In its substance are to be seen the *eggs* or *ova*, in varying stages of maturation.

The Excretory System. — The cavities and the external openings of the *nephridia* have already been noted. Such glandular tissue as is present is concentrated along the sides of the venæ cavæ.

The Nervous System. — Observe on each side of the animal, at a point on the inner surface of the mantle just anterior to the attachment of the retractors of the head, the large *stellate ganglia*. From these ganglia radiating *nerves* may be traced to different portions of the mantle and, running anteriorly from each, through the retractor of the same side, and thence to the *infra-œsophageal ganglion*, are the *right* and the *left pleural nerves*. Carefully trace these nerves to the cartilaginous support of the head, and then remove the tissue from the ventral side of the eyes until the enormous *optic ganglia* are exposed.

Lying ventrally, between the optic ganglia, and protected by cartilage, are the fused *pedal*, *pleural*, and *visceral ganglia*. They form the *infra-œsophageal mass*.

With a sharp scalpel carefully slice away the ventral cartilage, exposing the deep-lying nervous mass. Note, as the cartilage is being removed, the central cavities or *auditory chambers*.

The infra-œsophageal mass is prolonged anteriorly into the *pro-pedal ganglia*, which give rise to ten radiating

nerves which pass to the arms. The *siphonal nerves* are given off from the lower portion of the infra-œsophageal mass, while the *pleural* and *visceral nerves* arise more posteriorly.

From the smaller supra-œsophageal ganglion a pair of very minute connectives extend to the posterior surface of the buccal-mass, where they end, immediately over the œsophagus, in a pair of small *supra-buccal ganglia.* From the supra-buccal ganglia fibres extend around the œsophagus to the *infra-buccal ganglia.* The latter are of about the same size as the supra-buccal pair, and are united below the œsophagus by a commissure.

Make drawings of the ventral, lateral, and dorsal views of the brain.

THE LOBSTER[1]

(*Homarus americanus*).

External Anatomy. — Is the animal perfectly "bilaterally symmetrical"? What characters distinguish the anterior portion of the animal from the posterior? It will be observed that the latter, the *abdomen*, is segmented. Look carefully and see if there are traces of segmentation in the anterior portion, the *cephalothorax*. Is the number of abdominal segments constant?

Has each abdominal segment a pair of appendages? Do they all take their origin from similar points of the several segments? The ventral piece lying between each pair of appendages is called the *sternum*. The dorsal portion is the *tergum*. The ventrally projecting lateral lobes are the *pleurons*. The *epimeron* is a piece, more clearly seen on the first abdominal segment, that lies ventrally between the appendage and the pleuron. Note any specialization of any

[1] The lobster has been here introduced for several reasons. — Its generalized structure, large size, and the easiness with which its organs may be dissected make it a most convenient animal for the demonstration of not only arthropod morphology, but of general Invertebrate anatomy. It is, moreover, a typical crustacean, and in a shorter course might be studied as a representative of the entire Class. The crustacea considered farther on are especially valuable for comparative study.

abdominal segment and, if possible, give a reason for the same.

Is there anything to show that the last cephalothoracic appendage is homologous with the abdominal appendages? The latter have been called *pleopoda*. If the homology can be shown for the last, it must also be claimed for the remaining cephalothoracic appendages of equivalent origin and structure. How many such appendages are there? (Answer the question by comparing points of origin, number of joints, position of sensitive hairs, and number and position of protective spines. It will probably be concluded that there are five pairs closely resembling each other.) Why the remarkable enlargement of the anterior pair, the *forceps?* Do the five pairs of appendages arise from five segments? Will you admit of the "Rule of Savigny": "A pair of appendages represents a segment, though segments may occur without appendages"? How many segments, then, are there from the posterior end of the body to the forceps?

The appendages lying immediately anterior to the forceps are more or less specialized. They may be pressed down one after the other, like leaves of a book, and if found homologous with the appendages already examined, their number will indicate the number of supporting segments.

The most posterior, the *third maxilliped*, is in part like one of the ambulatory appendages already studied. Examine its point of origin, count its joints, etc. The immediately anterior, *second maxilliped*, is not remarkably different from the third, though the *first maxilliped* is considerably altered.

The line separating the maxillipeds from the anteriorly lying *second maxillæ* divides the head from the thorax. There are two pairs of maxillæ, the *second*, broad and leaf-like; the *first*, smaller and tricleft.

In front of the first maxillæ are the strong *jaws* or *mandibles*, each provided with a jointed accessory portion, the *palpus*.

Two pair of *antennæ* lie somewhat in front of the jaws. The *first antennæ* are shorter and biramous; the *second*, longer and with only a single filament.

The *compound eyes* are borne on stalks which are, by some, considered to represent appendages. Beginning with the eye-stalks, enumerate the segments of the entire animal.

Find the following openings: (*a*) The openings of the *auditory organ*, each situated at the base of the first antennæ and opening from the upper surface. (*b*) Of the excretory organs or *green glands*, on the lower side of the second antennæ. (*c*) The *mouth*, between the mandibles. (*d*) The *oviducts*, in females at the base of the third pair of ambulatory appendages. (*e*) The openings into the *receptive apparatus*, if the specimen is a female, in the angles of the heart-shaped structure between the next succeeding pair of appendages. (*f*) The *seminal openings* of the male, at the bases of the last pair of thoracic appendages. (*g*) The *anal opening* in the last segment (*telson*) of the abdomen.

Is there anything to show that some of the paired openings may be homologous?

Comparative Study of the Appendages. — Examine one of the appendages of the fourth abdominal segment. It will be found to consist of an elongated basal portion, the *protopodite*, bearing two blades, the *endopodite* within, and the *exopodite* without. Remove the left appendage where it joins the body and draw it as seen from in front, naming the parts. Compare with the appendages of the second, third, and fifth abdominal segments. Compare now with the first

and sixth. The appendages of the first abdominal segment are different in the two sexes. Do you find any trace of appendages on the terminal segment or telson?

Examine one of the thoracic appendages and endeavor to homologize the parts with the parts of the pleopoda. Pass to the third maxilliped and see if it has a protopodite, an exopodite, and an endopodite. Return now to the ambulatory appendage and see if you are better able to homologize its parts, so far as they are present, with parts of the abdominal appendage. (The ambulatory appendage is really an endopodite supported on a strengthened protopodite.)

Carefully remove the second ambulatory appendage of the left side and draw it as seen from the front, naming the parts. The proximal part of the protopodite bears a finely lamellate *gill* and a posteriorly directed membranous outgrowth, the *flabellum.*

Remove the remaining ambulatory appendages of the same side and see if all agree in structure. (Other gills situated higher up, on the epimera, will be considered later.)

Has the *third maxilliped* a gill? A flabellum? Remove, and draw as seen anteriorly, naming the parts.

After carefully removing the second maxilliped and noting the rudimentary condition of the gill, the reduced flabellum, and the number of divisions of the entopodite, draw as above directed.

The *first maxilliped* is without a gill, though its flabellum is remarkably developed. The flattened, elongated exopodite and endopodite extend anteriorly from the lateral angle, while the internal part of the protopodite is greatly enlarged and lobe-like.

Draw.

The *second maxilla* is even further altered: no gill is

present. The flabellum is indurated by a deposit of lime and stiffened by its fusion with the exopodite. What is the probable function of this the most anterior flabellum? The endopodite resembles the same of the first maxilliped. The protopodite is divided into four long inwardly-projecting lobes.

Draw.

After identifying the parts of the *first maxilla*, draw and name, taking care not to be confused by the fan-like outgrowths of the *lower lip*.

The body of each *mandible* represents the protopodite, while the palpus represents the endopodite.

Draw as seen from in front, naming the parts.

The *second antenna* presents a modification of the same double structure that has been noted in the other appendages. The protopodite bears an exopodite, a mere movable scale, and a much elongated endopodite, which functions as a tactile organ.

Draw.

The *first antenna*, the *antennule*, is in the adult also biramous, though primitively it is uniramous.

Draw.

Morphology of the Carapace. — It has probably been noted that a transverse groove divides the anterior portion of the cephalothorax from the posterior. The carapace, then, probably represents the fusion of terga, and our problem is to find out *which* have become fused.

If the groove be followed ventrally and anteriorly, it will lead to a septum between the second antennæ and the mandibles. It is probable, then, that the groove marks the dividing line between these two segments, second antennal

and mandibular. We know that in front of the groove three segments are represented, because related Crustaceans (*Squilla, Eupagurus, Gebia, Callianassa*) show these three segments more or less free, and in the lobster there are three pairs of appendages. It is probable that in the lobster the terga were also once free though now consolidated.

Elevate the free edges of the carapace and give reasons, if you have any, for considering them homologous with the pleura. Note the marginal hairs. Where are they most abundant? Their function? With a strong knife cut away all the right side which lies posterior and ventral to the groove. Note that the inner layer is membranous. Is it continuous at the margin with the outer layer? How far dorsally does the membranous part extend? Follow the line of its union with the epimera.

The space between the pleural piece and the epimera is the *gill* or *branchial chamber*. How many gills are there arising from the thoracic protopodites? Carefully remove these *podobranchiæ* with their associated flabella, keeping a record of their position. Above each exposed protopodite is a membranous *interarticular membrane* which bears the *arthrobranchiæ*. Record the number and position of the arthrobranchiæ. Above, are the four *pleurobranchiæ*. They belong to which segments?

Granting that the transverse groove marks the dividing line between two segments, it is reasonable to suppose that the part lying immediately behind it must represent a portion of the tergum and pleura of the mandibular segment. The fused thoracic epimera now being exposed, it is clear that the posterior portion of the carapace is certainly independent of the six posterior thoracic rings. To just which of the remaining segments, mandibular, maxillary or maxillipedal,

the posterior part of the carapace does belong is somewhat doubtful.

Internal Anatomy. — With a strong pair of shears carefully remove the calcareous shell from the dorsal half of the body, from the *rostrum* to the telson, noting the points where the muscles are attached. Have the attachments in any way affected the even contour of the exoskeleton? Examine still further the internal face of the removed portion of the carapace and see if there is anything to show that there are represented in it certain segments posterior to the groove.

Remove the dorsally lying pigmented *integument*, and also the superficial muscles, exposing the viscera. Note the large, anteriorly lying horny *stomach*. It is held in place by certain *gastric muscles*. Just back of the depression in the carapace formed by the groove, the *heart* will be seen. In outline it is somewhat shield-shaped, three angles being directed anteriorly and a single rounded angle projecting posteriorly. Two small, oblique openings will be noticed on its dorsal side, near the median line.

Do you find the elongated *sexual glands* extending on either side from near the lateral angles of the stomach to about the middle of the abdomen? Is the right gland in communication with the left? If the specimen is a female, the eggs may be seen through the transparent ovarian walls. Examine the outer border of the organ and find *ducts* leading ventrally.

Can you follow a blood-vessel, the *superior abdominal artery*, leading posteriorly from the heart and extending to the telson? Immediately below, and somewhat covered by the sexual organs, is the *intestine*.

The *liver* is the voluminous organ, of light green color, that fills up the greater part of the thorax. Note the segmental arrangement of the *thoracic* and *abdominal muscles.* Carefully remove the tissues at the base of the second antennæ, exposing the *green glands.*

Draw the animal as seen from above, using only the lightest outlines, as the details are to be put in after further study.

Remove the muscular and skeletogenous tissue from the lateral third of the left side of the body, thus giving a new view of the viscera. Lay the animal in water and note the boundaries of the organs already studied.

Probe through the mouth into the stomach. What direction does the short *œsophagus* take? The larger, more chitinous, anterior or *cardiac portion* of the stomach is separated from the posterior or *pyloric portion* by a groove running diagonally from the dorsal to the ventral line. Do you find that the insertion of muscles is in any way related to the hardened areas of the stomachic walls? Are there any pouch-like out-pushings of the pyloric portion? Is the pyloric portion chitinous? The whole organ resembles the exoskeleton and is, indeed, formed from an in-pushing of the outer covering of the body.

Pass to the posterior end of the alimentary tract and note that the *rectum,* the section lying behind the middle of the penultimate segment, is somewhat different from the straight *mesenteron* or *intestine,* which connects it with the pyloric portion of the stomach. Inject water through the anus and distend the intestine. Do you find a dorsally projecting sac, the *cæcum,* arising from the hinder end of the mesenteron?

Find the duct which leads from the left lobe of the

liver, or *mesenteric gland*, into the intestine. What is its extent? Cut the gland open and see if it has a lumen. Does the lumen communicate with the intestine? What is the plan upon which the organ is built up.

Carefully finish the drawing of the alimentary tract and its diverticula.

Remove the entire alimentary tract and float it out in clean water. Dissect away the anterior wall of the stomach and wash out the contents. Artificially work the *gastric teeth* together, thus demonstrating their function. Do you notice any apparatus that prevents the larger fragments of food from entering the delicate intestine? Do you find any organs which might, in function, distinguish between the nutritious and non-nutritious contents of the stomach? Are sensory hairs present in the œsophagus?

Slit open the intestine, cæcum, and rectum. Do you find any local differences in the lining membrane of the three portions?

Make a drawing of the interior of the right half of the stomach.

Excretory System. — With great care remove the left green-gland. Do you find the following parts: A smaller, ventral, *glandular portion*, of a deep green color in the living animal? A very large *saccular portion*, the delicate diverticula of which extend as lobes over the anterior and lateral walls of the stomach? The *urinary duct*, leading to the opening at the base of the second antennæ?

Reproductive System. — This may be worked out on the side of the animal which has not yet been injured. If the

specimen is a female the essential reproductive organs are the *ovaries*. Their position, color, and relation to the other viscera, should be clearly determined. How are they held in place? Can water be injected into them through the openings at the bases of the ambulatory appendages? Examine the *oviduct* and note its course. Are the eggs in different portions of the organ of about the same size?

Make a longitudinal incision through the ovarian wall and examine the interior. How are the eggs held in place? Is the wall of the oviduct in any way different from the same of the ovary? It is probable that it secretes a covering for the eggs, as they are being extruded, which attaches them to the hairs of the abdominal appendages.

Open the *receptive apparatus* and place some of the contained fluid on a microscope slide. Do you find, on examining the preparation with a high power, that *spermatozoa* are present? If the specimen which is being dissected measures ten inches in length, it will in all probability contain many of these *male-cells*, the structure of which is given below.

Draw the ovary.

Examine a male specimen. Note the position, color, and extent of the *testes*, the essential reproductive organs of the male. In what respect do they differ from the correlative structures of the female? Endeavor to inject through the external openings. Is the course taken by the *vasa deferentia* in any way different from that taken by the oviducts? Do you find the median portion of the vasa deferentia enlarged? Can you induce the extrusion of the contents (*spermatophore*) of this enlarged portion? Slit open one of the testes and place some of the contained fluid on a slide. Cover, and examine as above directed.

Each of the spermatozoa, or male-cells, will be found to consist of a somewhat cylindrical body, which is terminated at one end by a series of long spines.

Make a drawing of one of the testes as seen from the side, representing the vas deferens, and the external opening.

The Circulatory System. — The position of the heart has been already noted. In a specimen having the circulatory system artificially injected with a colored fluid, note the *pericardial sac*, a delicate tissue surrounding the heart. It receives the blood from the *branchiæ*,[1] which then enters the heart through six *cardiac apertures*. The *dorsal, lateral,* and *ventral apertures* occupy positions as suggested by their names.

The origins of the arteries, as they leave the heart, are as follows: —

The *superior abdominal artery* passes directly posteriorly from the heart, resting on the sexual organs, intestine, and deeper muscles. How many branches does it give off to each abdominal segment? Does it send branches to the sexual organs? Intestine? Cæcum? Note any terminal bifurcation.

The *sternal artery* has its origin from the most anterior point of the superior abdominal artery. It passes ventrally to supply the lower portions of the body. On which side of the intestine does it pass?

The *ophthalmic artery* runs from the heart anteriorly. Does it give off branches to the stomach? Can you determine its distribution in the anterior part of the head?

The *antennary arteries* arise from near the antero-lateral

[1] Later dissection will show that the blood is returned from the branchiæ through six *branchio-cardiac canals.*

angle of the heart. Each, in its course to the region of the antenna, gives off, besides others, a branch to the lateral walls of the stomach. Do you find the *renal branch*, distributed to the green-glands?

Draw in the circulatory system on the dorsal view of the animal already made, using a brush with light carmine paint.

Press to one side the viscera, which hide the duct of the mesenteric gland. A large artery, the *hepatic*, will be seen. From what part of the heart does it take its origin? Does it give off branches to other organs?

By the use of dots, rather than lines, designate its position on the drawing.

Turn the animal on its back, and through the transparent wall of the abdomen note the *inferior abdominal artery*. With a strong knife or with scissors, cut away the abdominal sterna, and see if the artery sends out metameric branches. Follow the artery anteriorly. It will be found to receive its blood from the sternal artery, which also gives rise to an anterior branch.

The *antero-ventral artery* passes along the mid-ventral line of the thorax. Name in order the branches that it gives off to the thoracic appendages. What is its anterior termination?

From the ultimate subdivisions of the main arteries, above noted, the blood passes into certain *sinuses*, and from these is sent to the subdivisions of the gills, through the *afferent branchial vessels*, and there purified. Does your specimen show the afferent vessels? After being purified, the blood from the minute gill-filaments empties into the *efferent branchial vessels* which unite to form the *branchio-cardiac canals*. The latter lead from the region of the thoracic epimera to the pericardium, where they empty

their contents. Does your specimen show the efferent branchial vessels? The branchio-cardiac canals?

In the injected gills note the course taken by the large *afferent* and *efferent vessels*. Is the flabellum also supplied with axial blood-vessels? Examine the gill with a lens and note the slender filaments.

Make a drawing that will show the structure of the gill.

The Nervous System. — The specimens have had many of the internal organs removed, but as the greater portion of the nervous system lies ventrally, it has not been injured. The system consists of a series of nervous centres, or *ganglia*, connected together by longitudinal and transverse commissures. Work out the following: —

The *supra-œsophageal ganglia* will be found beneath the rostrum at the base of the antennæ. The right is intimately connected with the left. Extending posteriorly and surrounding the œsophagus, are the *circum-œsophageal commissures*, which are soon united by

The *infra-œsophageal ganglion* which lies immediately behind the œsophagus.

From the infra-œsophageal ganglion the *longitudinal commissures* or *connectives* may be followed to the region of the telson.

How many thoracic ganglia are there? How many abdominal ganglia? Are the ganglia of one series more closely approximated than those of the other? Does the number of ganglia in anyway correspond with the number of associated body segments? Do you find nerves running from the ganglia to the surrounding tissues?

Make two drawings of the nervous system, one as it is seen from above, the other as it is seen from the side.

A PHYLLOPOD CRUSTACEAN

(*Branchipus vernalis*).

External Anatomy. — Observe the elongated *body*. Is it distinctly segmented? Is it separated into a *head*, a *thorax*, and an *abdomen?* The *appendages* are leaf-like (Phyllopod). They occur on which main division of the body? Are they represented on the other regions? Observe the pair of flexible *anterior antennæ*. In the males the *second antennæ* are enormously developed and form "clasping organs." Large at the base, each second antenna soon becomes constricted, and forms an elongated chitinous rod. Find the homologues of the second antennæ in the female.

The upper lip, *labrum*, will be found as a median fold extending posteriorly from between the bases of the second antennæ. Elevate the labrum and note that it covers the *jaws* or *mandibles*. In what plane do the mandibles move? Are the cutting edges specially hardened? Note the opening of the *mouth*. The *maxillæ*, relatively poorly developed, lie immediately posterior to the mouth.

How many pairs of *thoracic appendages* are there? Carefully remove one of the first pair, and note that there is a basal portion, *protopodite*, which is extended distally by certain lobes. The internal lobe, *endopodite*, is the largest and bears numerous *setæ* along its inner and distal edge. Of the same general structure is the outer lobe or *exopodite*, which

bears several longer setæ. External to and above the exopodite is a small sac, the *gill-sac*. Above the gill-sac is the broad, paddle-shaped *gill*.

Examine the remaining thoracic appendages. Are they all built upon the same plan? What functions do they perform? Recall the parapodia of *Nereis*.

At the anterior end of the abdomen are the *external sexual organs*. In the female they will be seen to enclose the *eggs*. In the male a tubular organ is formed. Are the terminal fin-like organs comparable with the thoracic appendages?

Make drawings of the specimen as seen from the side, and of the more important and characteristic parts.

Digestive System. — Open a specimen along the mid-ventral line, and observe the course of the alimentary tract. It extends as a tube from the mouth to the *anus*. Does the anus open dorsally or ventrally? Note any local enlargements of the alimentary tract.

Make a drawing of the tract.

The Circulatory System consists of an elongated dorsally lying tube, the *heart*. It is provided with numerous lateral *slits*, a pair for each surrounding segment, and extends from near the anterior end of the thorax to the posterior part of the abdomen. The blood enters the heart through the lateral slits, and is forced out through the *anterior aorta* into the sinuses of the body, finally reaching the lamellated gills, where it is aërated. After being purified, a system of channels conveys the blood to a *pericardial sinus*, from which it passes through the slits or *ostia* into the heart.

The Excretory System. — A pair of so-called "*shell-glands*" may function as renal organs, and have been compared with *nephridia*. They open to the exterior through two papillæ, near the bases of the second maxillæ.

The Muscular System is well developed. Observe the extensor and retractor muscles of the abdomen. Also note, in the thorax, the presence of regularly arranged muscle bundles. Recall the arrangement in *Nereis*.

The Nervous System. — Observe the minute, median *ocellus*. Below and on each side of it are the first antennæ, the tactile organs. The *compound eyes* are large and prominent. Are they *stalked?* Examine carefully and note the *facets*.

Make a drawing of the head, paying particular attention to the organs of special sense.

Carefully remove the appendages from another specimen, using a pair of fine scissors. Cut the animal open along the mid-dorsal line, and remove the lateral walls of the thorax and abdomen, together with all but the most anterior portion of the alimentary tract. Wash out the cavity with ninety-five per cent alcohol, and then place in absolute alcohol for one quarter of an hour. Remove from absolute alcohol and place in xylol. The delicate *ventral chain of ganglia* will soon take on a different appearance from the rest of the specimen. Note that there are two ganglia in each thoracic segment, each connected with its fellow by a *cross commissure*. The ganglia lying anterior and posterior to each other are united by *longitudinal connectives*. Follow the chain into the abdomen and also anteriorly into the head. As in *Nereis*, it will be found to surround the œsophagus and

to pass above into the *supra-œsophageal ganglion* or *brain.* Trace the nerves from the brain to the *ocellus* and to the *compound eyes.*

Make a drawing of the nervous system.

The Reproductive System. — The "brood-pouch" of the female has already been noted. The *ovaries* are paired organs, each lying parallel to the alimentary tract, and extending through a considerable portion of the thorax and abdomen. They are beautifully brought out by the adoption of the xylol method above described. Note, in the brood-sac, the large *eggs.* Do you find any pore through which the eggs may pass from the sac to the exterior?

Make drawings of the female reproductive system.

The *external reproductive organs of the male* are situated on the anterior portion of the abdomen. Each consists of a somewhat complicated apparatus, which is used in placing the *spermatophores* in the sacs of the female. The *testes* will be easily found as paired organs, of the same general structure as the ovaries, though of not equal extent.

Draw the reproductive system of the male.

A FREE-LIVING COPEPOD

(*Cyclops sp.*).

SELECT a large female specimen (the females generally have a pair of *ovisacs* suspended from the body), and examine under a compound microscope. The *body* will be found to be well rounded, neither *depressed* nor *compressed*, considerably elongated, and clearly segmented. The divisions into *cephalothorax* and *abdomen* are at once apparent. The former consists of an anterior, unsegmented *carapace*, behind which are four movable thoracic segments. Has the carapace a *rostrum?* Are the edges of the carapace free? Are the several thoracic rings all of about the same size?

In the present sex the abdomen has four segments, though the first is in reality formed from two. Do you find anything to suggest that fusion has actually occurred? In the smaller male there are five abdominal rings, the original structure being retained.

How many filaments are there arising from the terminal *abdominal styles?* Are there *thoracic appendages? Abdominal appendages?* Note the position of the enlarged *first antennæ*. Are they provided with hair-like *setæ* (olfactory hairs)? Compare them with the smaller *second antennæ*. In the males the antennæ are reduced in size and functionize as *clasping organs*.

Examine the *swimming appendages* of the posterior por-

tion of the cephalothorax. Four well-developed pairs will be found, each consisting of a basal portion and two free terminal blades. The fifth pair are reduced. The *mouth parts*, consisting of a pair of *mandibles* and two pairs of *maxillæ*, are too small to be profitably studied at this time.

Digestive System. — Specimens that have been feeding upon some colored substance (carmine) should be selected. They may be rendered inactive by placing a drop of ether in the watch-glass that contains them. The *mouth*, situate on the lower surface, just behind the origin of the antennæ, will be found to lead into a large, somewhat thick-walled *crop*. From the crop the *intestine* extends in a direct course to the *anus*. Does the anus occupy a dorsal or a ventral position? Through which abdominal segment does it open?

Circulatory System. — Though in certain related forms there is a dorsally situated contractile *heart*, in the present form, and, indeed, in the greater number of Copepods, rhythmic contractions of the entire alimentary tract produce the circulation of the surrounding *cœlomic fluid*.

Excretory System. — A pair of coiled tubes, the *shell-glands*, occupy lateral positions in the thorax and open to the exterior through pores at the bases of the maxillipeds. They are only to be seen in specially favorable specimens. The intestine probably has some excretory power, while the entire surface of the body may permit the escape of injurious gases, combining excretory and respiratory functions.

The Muscular System is somewhat difficult to demonstrate. It consists of a series of regularly arranged fibres,

which bring about the movement of the second antennæ, and other less important locomotor appendages. There are also two large muscular bands that forcibly flex the body.

The Nervous System is too difficult of demonstration to be here considered. The *olfactory hairs* of the first antennæ have been noted. The *ocellus* is the visual organ.

The Reproductive System. — In the female the *ovary* is found to occupy a median dorsal position, extending as a somewhat elongated organ about two-thirds the length of the cephalothorax. Leading from the ovary to the external ovisacs are the *oviducts*. Each oviduct is a coiled structure, lying laterally to the ovary and often containing *ova*. Its external opening is through the first abdominal segment. The ova are fertilized from a *receptaculum seminis*, as they pass into the large ovisacs. How many eggs does each ovisac contain? The young hatch in the interesting "Nauplius stage."

The sexual organs of the male consist of a median *testis* and two coiled, laterally extending *vasa deferentia*. Each vas deferens finally opens into a terminal receptacle, where the spermatozoa are collected, and form a *spermatophore*. At time of coitus the spermatophore leaves the body of the male, through an opening in the first abdominal segment, and passes into the *receptaculum seminis*.

Make drawings of the dorsal and lateral views of the male and female.

A PARASITIC COPEPOD

(*Pandarus sinuatus*).

THE species under consideration leads a parasitic life upon the external surface of the shark. Only females have been collected. Parasitism has wrought many interesting changes.

External Anatomy. — When compared with the free-living Copepod, *Cyclops*, the present form will be found to have the *body* depressed, the *cephalothorax* enlarged, and the posterior portion of the *abdomen* somewhat reduced. Compare the general outline with that of Cyclops. Is a *rostrum* present? Observe the location of the *visual organ*, and compare with *Cyclops*. Note the outline of the posterior three thoracic segments. The first abdominal segment is greatly enlarged, and within its transparent walls the coiled *ovary* and *oviducts* are to be seen. A rounded, flap-shaped piece terminates the body posteriorly.

Make an enlarged drawing of the dorsal view.

Examine the ventral surface and note its concavity. The *first antennæ* appear as free, blade-like organs along the most anterior edge of the cephalothorax. Do they have flexible tips? Are they provided with *sensory hairs?* At the base of each is a *sucking disc*.

Having their origin nearer the median line, and bearing, laterally and posteriorly, a pair of suckers, are the small, hook-shaped *second antennæ*. What is their function?

The *sucking proboscis*, which is in part made up of the greatly modified *mandibles*, arises as a styliform organ, midway between the second antennæ. It surrounds the *oral opening*.

The *first maxillæ* are very much reduced in the parasitic crustacea. In the present form they appear as a pair of minute palp-like organs, situated laterally on the flaring base of the proboscis.

The *second maxillæ* are well developed, and appear as a pair of long, slender-jointed appendages arising posterior to the second antennæ. Their free extremities are seen to be provided with hooks, while a pair of suckers will be found between their points of attachment.

The *maxillipeds* are considerably larger, directed posteriorly rather than laterally, and bear, at their free ends, a peculiar attachment organ. Behind the maxillipeds are four pairs of *swimming appendages*, of which the first are somewhat reduced. Note the median sucker, between the first and second pair of swimming feet.

The body is terminated below by a flap-like segment that bears a pair of laterally projecting *styles*. Compare with *Cyclops*.

The greatly elongated *egg-strings* will be found to take their origin laterally from a point midway between the dorsal and ventral terminal flaps.

Make drawings of the ventral surface and of the several appendages.

Nauplii, the free-swimming young of many crustacea, may be secured from the present form and in large numbers. Tease one of the egg-strings and place its contents under a compound microscope. Each Nauplius will be found to bear three pairs of jointed appendages, which repre-

sent the *first* and *second antennæ* and the *mandibles* of the adult. Are the appendages biramous? Is the body of the Nauplius segmented? Are there posteriorly directed terminal styles? Is there a median *ocellus?* Can you trace out the *alimentary tract?*

Make a drawing of the Nauplius.

A CIRRIPED CRUSTACEAN

(*Lepas fascicularis*).

External Anatomy. — The highly specialized animal is attached at its *anterior* end by an elongated *peduncle*, and is protected laterally by a mussel-like shell. Of how many pieces does the shell consist? Do you find an elongated opening between the two halves of the shell? The opening is on the ventral side. The median, dorsal piece of the shell is the *carina*, while lying laterally are the more posterior *terga* and the more anterior and ventral *scuta*. The scuta join in the mid-ventral line. From the ventral opening the six pairs of biramous appendages are thrust out for the securing of food.

Draw the animal as seen from the right side, naming the parts.

Stretch apart the valves and note that the integument of the animal, externally indurated with lime, passes, within the ventral opening, into a more delicate transparent fold, which completely separates the soft parts of the animal from the exterior. Note in particular the deeply lying *oral cone*,[1] the attachments of the *biramous appendages*, the aborted *abdomen*, and the greatly elongated *intromittent organ*, which occupies a position just back of

[1] The oral cone is a large knob-like mass which is made up of the mouth-parts, and bears, at its apex, the mouth.

the last pair of thoracic appendages.[1] Do you find the *anus?*

Remove and make a drawing of one of the appendages.

Before the body has been opened, the transparency of the integument permits the location of several internal organs. A large dark-colored area on either side, near the base of the cone, marks the position of the "*hepatic glands.*" A smaller, orange-colored area near the base of the most anterior filament is the anterior end of the *vas deferens.* On the ventral side the muscular apparatus and the *ganglionic* chain are beautifully shown.

Carefully remove the right half of the shell, and trace the folds of the integument into the peduncle. The peduncle is a prolongation of the head, the *anterior* (*first*) *antennæ* of the larva having entered into its formation.[2] The *second antennæ* are, in the adult, aborted.

At the summit of the oral cone a large transverse fold will be found to bound the mouth anteriorly and to bear at each end a triangular "scale-shaped" piece. The *mandibles* and *first maxillæ* bound the mouth laterally, while the *second maxillæ* are partially fused and form a lower lip. Both mandibles and first maxillæ have strong cutting edges.

Digestive System. — Carefully opening the animal along the lateral line, note that a funnel-shaped tube leads from

[1] The abdomen is greatly reduced. It is represented on the dorsal side by a pair of small terminal flaps which cover the anal opening, while on the ventral side it supports the intromittent organ.

[2] The first antennæ bear the so-called "cement glands," the secretion of which enables the larva to attach itself. In the adult these glands are to be found in the substance of the peduncle occupying a position in its dorsal portion.

the *mouth* to the short, straight *œsophagus*. The latter opens suddenly into a large *stomach*. Numerous dark *hepatic glands* will be seen on the walls of the stomach. The *intestine* is a straight tube of uniform size, extending from the stomach to the anus.[1]

Make a drawing of the alimentary tract as seen from the side.

Excretory System. — Glandular bodies, possibly excretory in function, have been described as opening from the second maxillæ. The *cement glands* of the first antennæ may also belong to the category of excretory organs.

A closed **Circulatory System** is not present in the Cirripedia. Near the base of the oral cone, on either side of the animal, five elongated *filaments* are to be noted. These organs are probably respiratory in function.

The Muscular System is developed chiefly for the movement of the divisions of the shell, the waving of the appendages, and the retraction of the soft parts. The young (Nauplii) are active swimmers.

The arrangement of the muscles that move the mouth parts can often be seen through the transparent integument of the oral cone.

The Nervous System. — Remove the stomach and intestine from the opening already made in the side of the animal. The nervous system occupies, as a chain of ganglia, a median ventral position. Anteriorly it surrounds the œsophagus (*œsophageal ring* or *collar*), and unites with the

[1] On either side of the œsophageal end of the stomach is a whitish glandular organ, which may be called the "pancreas."

supra-œsophageal ganglion, from which nerves pass to a pair of minute, pigmented *eye-spots*.

Draw.

The Reproductive System. — The barnacle is hermaphrodite. The *ovaries* will be found in the peduncle, the *ova* appearing as a collection of minute spheres loosely held together. The *oviducts* pass from the ovaries to the bases of the anterior thoracic appendages, and are difficult to follow.[1]

The *testes* may be seen through the transparent integument of the body and appendages. Each gland, in alcoholic specimens, appears as a racemose structure of pale yellow color, the lobes extending from a larger antero-dorsal mass into the more anterior thoracic appendages, and into the filaments.

If the six pairs of thoracic appendages be spread apart, the *vasa deferentia* will be observed entering the base of the intromittent organ. These ducts will be found to lead from the anterior end of the testis and to unite in the penis and form a common *ductus ejaculatorius*.

Make a drawing of the reproductive system.

[1] The opening of the oviduct is on the anterior surface at the point where the appendage joins the body.

A BRACHYURAN CRUSTACEAN

(*Cancer irroratus*).

External Anatomy. — Are the *cephalothorax* and *abdomen* built upon a new plan, or is the plan presented by the lobster again adopted? Which region of the body has been specially elaborated? The *abdomen* of the female is much wider than that of the male; to which sex does the specimen belong?

Count the abdominal appendages of a female, and see if they agree with the same of the lobster. Count the same of the male. Why are there more appendages on the abdomen of the female than on that of the male? (Examine a specimen that bears eggs.) Is the number of abdominal somites different in the two sexes? Does the male show any traces of coalescence of abdominal segments? How does the number compare with the abdominal segments of *Homarus?*

Is the number of *ambulatory appendages* the same as in the lobster? The number of appendages bearing pincers? In what main feature does the *carapace* differ from the carapace of the lobster? Do you find the homologue of the *rostrum?* The *transverse groove* is more clearly shown in many related forms. Can you easily follow the groove to the margin of the carapace? Is this a free margin, as in *Homarus?* Note the opening at the base of the first ambulatory appendage, through which the water enters to bathe

the *gills*. Do you find the external openings of the *oviducts*? Of the *vasa deferentia*?

Draw the animal as seen from above.

Remove the lower portion of the left thoracic pleuron, and expose the bases of the appendages. *Podobranchiæ* and *arthrobranchiæ* are not present. Are there *flabella* at the bases of the ambulatory appendages?

Carefully remove the *third maxilliped* of the left side. Compare it with the same of the lobster.

Draw.

The *second maxilliped* will be found to have *endopodite*, *exopodite*, and *flabellum*, of the same general structure as in *Homarus*. Only two *gills* are present.

Draw.

The *first maxilliped* is foliaceous and gill-less. Note its greatly elongated *flabellum*, which extends far back into the *branchial chamber*.

Remove the appendage and draw.

Examine the right (uninjured) side, and see if you do not find at the base of the first maxilliped, and extending anteriorly, a large opening that leads from the branchial chamber.

The *second maxilla*, as in the lobster, has a specially developed flabellum for bailing the water out of the branchial chamber. Note how its protopodite is divided almost precisely as in *Homarus*. The rudiment of the endopodite is especially interesting.

Draw.

Remove the *first maxilla* and draw, and do the same with the *mandible*.

Examine the *antennæ*, and see which are *first* and which *second*.

Remove the second antenna. Do you find the elongated filament that was specially developed in the lobster? Can you find the openings of the *green-glands?*

Draw a second antenna.

Carefully remove the *first antenna*. Find the position of the *auditory sac*. Do you find the representatives of the terminal filaments of *Homarus?* Which filament is provided with numerous sensory hairs?

Draw.

Do the *compound eyes* differ from the same of the lobster?

Draw.

Without injuring the gills, remove the right branchial wall. *Pleurobranchiæ* will be found on the fifth and sixth thoracic segment; *arthrobranchiæ*, one on the second, two on the third, and two on the fourth; while *podobranchiæ* are found only on the second and third thoracic segments.

Remove one of the gills, and see how its structure differs from that of the gill of the lobster.

Internal Anatomy. — Carefully remove the dorsal portion of the carapace. Cut away the underlying integument, and expose the viscera. Cover the specimen with water, and, without displacing or injuring the organs, note as follows: —

The *heart* and its two pairs of *cardiac apertures* or *valves;* the *pericardial sinus;* the openings into the sinus of the *branchio-cardiac* canals; the *ophthalmic artery;* the *antennary artery;* the *hepatic artery;* the *superior abdominal artery.*

Somewhat anterior to the heart lies the *stomach*. Compare with *Homarus*. Note the arrangement of the dorsal

gastric muscles. The lobes of the *liver*, or *mesenteric gland*, extend laterally until they reach the limits of the shell. Each lobe, as in the lobster, is composed of a multitude of *tubules.*

Lying anteriorly to the heart, and extending laterally over the mesenteric gland, are the voluminous *sexual organs.* The *testes* appear as a coiled filament, extending from near the lateral walls of the stomach to the lateral angles of the shell. In the region of the pyloric end of the stomach the filament enlarges into a coiled duct, the *vas deferens.* Expose the *green-glands*, and trace out their ducts.

The comparison of the *nervous system* of the Brachyuran with that of the Macruran (Homarus) will prove most interesting.

Draw the animal as seen from above.

AN AMPHIPOD CRUSTACEAN

(*Talorchestia longicornis*).

External Anatomy. — Select a large male specimen. (The males are generally larger and have antennæ which almost equal the body in length.) Note the laterally compressed body and the numerous jointed appendages. Distinguish between the *head*, the *thorax*, and the *abdomen*.

Projecting anteriorly from the head are the smaller *first antennæ*. How many joints has each? Immediately beneath the first antennæ are the long *second antennæ*. Of how many joints does each consist? Compare with the same organs of the female.

Remove the head and examine from the side. Are there any suture-like lines that might lead one to suppose that the head is really composed of several fused segments? Note the lobe-like upper lip or *labrum*. In what plane does it move? Is there a somewhat similar organ (the united *first maxillipeds*), covering the mouth-parts from behind?

Remove both labrum and the fused maxillipeds. The remaining mouth-parts move laterally.

Lying immediately anterior to and under the maxillipeds are the *second maxillæ*. Each is a delicate, foliaceous, bilobed organ, and bears numerous setæ along its free edge.

Remove the second maxillæ, and expose the more rigid bilobed *first maxillæ*. The large, irregular *jaws* work between the *first maxillæ* and the labrum.

Open the jaws and note the opening of the *mouth*.

Make drawings of the several appendages of the head.

The first thoracic segment, bearing the first maxillipeds, has, as already noted, become fused with the head. The second thoracic segment bears a pair of fair-sized second maxillipeds, while the third, homologous with the third maxillipeds of the lobster, bears a pair of enormous *chelæ*. Behind the chelæ are five pairs of ambulatory appendages supported on as many separate thoracic rings.

Note the expanded *coxal joints* of the thoracic appendages, the *epimeral plates*. Spread the appendages apart, laterally, exposing the *gills*. Each gill arises from the posterior side of an appendage, near where the latter is inserted. How many gills are there? Do you find the same number in the female? In the latter do not confuse the gills with the *oöstegites*, or *lamellar plates*, which form the *brood-pouch*.

Attached to the anterior part of the abdomen are three pairs of *swimming-feet*, while the posterior portion bears the same number of *jumping-feet*. Are there six separate segments bearing the six pairs of appendages? Note the small *terminal plate*. Are all the abdominal appendages biramous?

Digestive System. — Remove the appendages from a large specimen, and, after opening along the mid-ventral line, pin the animal out under water or alcohol, and wash any cloudiness away.

The short *œsophagus* will be found to lead into a somewhat rounded *crop*, from which extends, posteriorly, the straight *intestine*. The *hepatopancreatic cœca* appear as four elongated

sacs extending from their attachment at the anterior end of the intestine, two-thirds the length of the body. How are they held in place? Does the *anus* open dorsally or ventrally?

Draw.

Excretory System. — A pair of small, somewhat coiled diverticula, the *mesenteric tubules*, are to be seen lying along each side of the more posterior portion of the intestine. Trace out their course and see where they open into the intestine. The external openings of the *antennal glands* may be observed, near the median ventral line, as two small pores, each on the basal joint of a second antenna.

Draw, as seen from the side.

The Reproductive System. — The *testes* are to be observed as a pair of delicate tubes lying longitudinally, immediately above the middle portion of the intestine. Follow both to their external openings. Are the testes in the thoracic or in the abdominal cavity?

Draw.

The *ovaries* occupy a position similar to that of the testes. The *openings of the oviducts* may be found at the bases of the fifth pair of appendages. Each is a small pore between the gill and the oöstegite.

Draw.

The Circulatory System. — The *heart* will be found as a delicate elongated sac lying along the dorsal part of the thoracic cavity. The blood enters the organ through certain lateral *ostia* and is then forced, through *anterior* and *posterior aortæ*, into the various lacunæ of the body. The blood finally reaches the *gills*, situated, as has been observed, on the thoracic segments. It is from them returned to the heart.

The Muscular System. — The Amphipods are active jumpers. Observe the strong extensor muscles of the tail. Determine also the general arrangement of the muscles which move the appendages.

The Nervous System. — What is the position of the *compound eyes?* Are they *stalked* or *sessile?* Certain specimens clearly show, through the transparent sterna of the thorax and abdomen, the ventral *ganglionic chain.* Is it clearly made up of two strings? Do you find *longitudinal* and *transverse commissures?* Do nerves leave the *ganglia?* Does the number of ganglia correspond to the number of segments? Carefully dissect in the region of the head, noting the *infra-* and *supra-œsophageal ganglia* and the *œsophageal ring* or *collar.*

Make a drawing of the nervous system, naming all the parts.

THE KING CRAB

(*Limulus polyphemus*).

External Anatomy. — Note, on examining the animal from above, that the chitinous shell is divisible into a large anteriorly lying *cephalothorax* and a smaller, polygonal *abdomen*, terminated posteriorly by a rigid *caudal spine*.

The cephalothorax bears a pair of lateral, *compound eyes*, and a pair of median *ocelli*. The latter occupy positions, one on each side of the frontal spine.

Is there anything on the dorsal surface of the abdomen that would show that it might possibly be made up of several fused *segments* or *metameres?* How many *movable spines* are there along the margin? How many pairs of *pits* each side of the median line?

Draw.

Examining the lower, concave surface of the specimen, observe the seven pairs of *cephalothoracic limbs*. The first (*cheliceræ*), smaller than the remaining, are followed in immature specimens and in females by four pairs of pincer-bearing *legs*. Do the pincers open and close as in *Homarus?* The sixth pair of appendages are directed posteriorly. Have they anything that might be of assistance in forcing the animal through the mud? Note the exopodite-like process at the base of each.

The *chilaria* are supposed by some to represent a pair

of appendages. They are small unjointed processes lying between the bases of the pair of appendages just considered.

The *seventh pair* of cephalothoracic appendages are considerably modified and form the *operculum* or cover for the abdominal appendages. The two are united in the median line. Certain transverse lines mark their lines of original segmentation.

The *mouth* opens in the median line between the bases of the four pincer-bearing appendages. In front of it is a conical elevation, the *upper lip*.

The abdomen bears five pairs of modified appendages, each pair resembling the operculum, and covering the large *gill-books*. By the movement of the abdominal appendages and the gill-books the animals can slowly swim through the water.

At the base of the caudal spine the opening of the *anus* will be observed. The *external openings of the genital organs* will be found on the posterior surface of the operculum. The external openings of the *coxal glands* have been found in embryos on the basal joints of the fifth pair of cephalothoracic legs.

Internal Anatomy. — With a strong knife remove the entire upper portion of the shell of both cephalothorax and abdomen.

The Muscular System. — The strong *flexor muscles* will be seen to have a longitudinal course along each side of the median plane. The muscles which move the legs are disposed laterally to the flexors, and their fibres are arranged, for the greater part, dorso-ventrally.

The Circulatory System. — Along the mid-dorsal line, just below the flexor muscles, the elongated *heart* will be

found. It extends from a point under the median eyes to the posterior half of the abdomen. It has, extending anteriorly, a *frontal artery* which divides into a right and a left *anterior marginal artery*.[1] The marginal arteries bend outwardly, laterally, and posteriorly, following the curve of the margin of the cephalothorax. The *posterior marginal arteries* are the abdominal continuations of the anterior marginals. Extending laterally from the heart are the *lateral arteries*.

The "*aortic arteries*" take their origin from the anterior end of the heart, and pass, one on either side of the œsophagus, to the *circum-oral reservoir*, below mentioned.

The *first lateral arteries* branch off from about the middle of the cephalothorax; the *second* and *third lateral arteries*, from the posterior portion of the cephalothorax. From the second, branches communicate with the marginal arteries and with the *hepatic arteries*. The latter lie parallel to the anterior marginal, and supply blood to the liver.

The *collateral arteries* lie longitudinally, one on each side, parallel to the heart. They connect the several lateral arteries.

The heart is continued posteriorly by the *superior abdominal artery*.

Branchial arteries pass from the abdominal collaterals to the gills.

The arteries of the ventral side cannot be now dissected. They enclose the nervous system and consist of a *circum-oral reservoir*, from which the *crural arteries* are given off to the legs. Extending posteriorly from the reservoir is the *ventral* artery, which gives rise to *branchial* and *anal* branches.

The blood is returned from the gills and all parts of the

[1] The frontal artery extends anteriorly to the margin of the cephalothorax before dividing into the marginal arteries.

body, through certain irregular channels, to the pericardial sac, from which it passes into the aorta through a series (eight pairs) of *ostia*.

Make drawings illustrating the course of the heart and principal blood-vessels.

The Digestive System. — Remove the heart and expose the more ventrally lying alimentary tract. The mouth will be found to lead into an anteriorly and upwardly directed *œsophagus* which opens into a strong-walled *anterior stomach* or *crop*. Cut the crop open and note the character of its lining. The crop opens posteriorly into a thin-walled *stomach*, which extends, without any remarkable change, to the short *proctodœum* or *rectum*, the latter opening to the exterior through the anus.

Carefully open the stomach and note the entrance of the *bile-ducts*. These bile-ducts lead from the voluminous *liver*, which nearly fills the cavity of the cephalothorax.

Make a drawing of the digestive system.

The Reproductive System. — The sexes are separate. The *ovaries* or *testes* form a network of tubes which ramify over and through the substance of the liver. Their ducts may be traced to the external openings already noted.

The Excretory System. — The *coxal glands* lie along both sides of the stomach, each consisting of a longitudinal portion, from which project laterally four finger-shaped lobes. In life they are "brick-red" in color. The function of these organs is not definitely known.

Draw.

The Nervous System. — Remove the alimentary tract from the beginning of the crop to the anus, and expose,

immediately under the stomach, the cartilaginous *sternal plate*. Note the outline of the plate and remove it with care, as a portion of the nervous system lies immediately beneath.

The nervous system consists of a *brain*, a *circum-oral ring*, and a ventral, posterior continuation, the *ventral cord*. The *brain* is a rounded body lying in front of the œsophagus. Trace the nerves leading from it. The circum-oral ring is a flattened disc which innervates the ambulatory appendages. The posterior part of the ventral cord gives off branches to the abdominal appendages. The more central portions of the nervous system are surrounded by a blood-sinus.

Make a drawing of the nervous system.

AN ARACHNID

(*Epeira riparia*).

External Anatomy. — The *body* is here again divided into a *cephalothorax* and an *abdomen*, the two being united by a small *peduncle*. The cephalothorax is flattened above and bears six pairs of appendages. The most anterior pair, the *cheliceræ*, are provided with strong cutting jaws and are pierced, near their tips, by a small opening which leads from a *poison-gland*. They lie anterior to the *mouth*.

The *pedipalps*, which lie immediately behind the cheliceræ, and on each side of the mouth, are more leg-like. They consist of a strong-bodied, basal portion and of an elongated, many-jointed *feeler* or *palpus*. In the males the palpi are specialized into organs for bearing the *spermatophores*. Compare this pair of appendages with the same of *Limulus*.

The four pairs of *legs* take their origin from behind the mouth, along the lateral portion of the cephalothorax. Do they all agree in having the same number of joints? Note the hook-like organ terminating each.

Examining the abdomen from above, do you find anything (grooves or transverse bands) that might suggest that it was once segmented?

The lower surface of the abdomen presents several organs of interest. — The *external openings of the lung-sacs* appear as two transverse slits, one on either side of the anterior portion of the abdomen. In the median line, between them, is

the *external opening of the sexual organs*, in the female highly elaborated. The *spinnerets* will be found at the posterior end of the abdomen as three pairs of papillæ. They are jointed, and probably represent specialized abdominal appendages. The smaller middle pair of spinnerets are covered by the much larger anterior and posterior pair, while the *anal papilla* is closely folded against the latter as a transverse chitinous elevation. If the anal papilla is pressed back, the opening of the *anus* will be found.

A small pore, or *spiracle*, lying in the median line, immediately in front of the spinnerets, leads from a series of branched *abdominal tracheæ*. This pore is often difficult to find. It lies just in front of a thorn-like process which rests just between the first pair of spinnerets. If the integument at this point be carefully picked away, the tracheæ will be found.

Make a drawing of the spider as seen from above, and another as seen from below.

Internal Anatomy. — Carefully remove the integument from the upper half of the thorax and abdomen.

The Circulatory System. — The *heart* is an elongated, thin-walled tube which extends along the dorso-median portion of the abdomen and is continued anteriorly, as the *anterior aorta*, through the peduncle, into the cephalothorax. The heart is really enclosed in a very delicate *pericardium*, which holds the blood as it is returned from the *lacunæ* of the body, finally pouring it into the heart through certain lateral *ostia*. The blood, leaving the heart through the anterior and posterior aortæ, is conveyed in arteries for only a short distance. It soon enters lacunæ and sinuses, in which it pursues a definite course until it gathers in a large reservoir

near the lung-sacs. In the lung-sacs it is purified, and from thence passes directly to the pericardium.

The Reproductive System. — The *ovaries* of the female often nearly fill the abdominal cavity, the *ova* being very irregular in outline, as a result of their mutual pressure. The *oviducts* may be traced to the external opening already noted. Lying on either side of the external opening is a coiled tubular organ, the *receptaculum seminis*, that receives the spermatophore of the male, and retains the *spermatozoa* until the time of egg-laying. The *testes* of the male are elongated tubular glands, which lie longitudinally along the lower portion of the abdominal cavity. Before opening to the exterior each gland is continued into a coiled *vas deferens*.[1]

The Digestive System. — The straight *intestine* will be found to lie immediately under the heart and to give off laterally several *cœcal diverticula*. Followed posteriorly, the intestine opens into an enlarged *rectum*, which also receives the ducts from certain many-branched tubes, the *Malpighian vessels*.

Followed anteriorly, the intestine passes through the peduncle and enlarges within the cephalothorax into a *stomach*. From the stomach, cœcal diverticula extend out as loops into the bases of the legs. Certain *gastric muscles* are so arranged that they can enlarge the central portion of the stomach, which thus acts as a sucking-organ.

The *œsophagus* may be followed as a tube leading from the mouth to the stomach.

[1] The spermatic fluid is collected in the pedipalps and retained in spermatophores until transferred to the receptacula of the female.

The *poison-glands*, which are probably modified salivary glands, will be observed in the anterior portion of the cephalothoracic cavity, immediately over the cheliceræ. Trace their ducts to the terminal openings already noted.

Make an imaginary drawing of the viscera, thus far studied, as they would appear in side view.

Carefully remove the digestive tract and note, lying immediately below it in the cephalothorax, the "diaphragm," a thin sheet of tissue extending horizontally almost entirely across the cavity. Recall the sternal plate of *Limulus*.

The Respiratory System. — The external openings of the lung-sacs have already been noted. Each *lung-sac* contains a cavity into which a series of *lamellæ*, like the leaves of a book, project. In these thin-walled lamellæ the blood circulates and is purified. Recall the gill-books of the king crab.

The *spiracular opening* of the tracheal system of air-tubes has been already noted. The arrangement of the *tracheæ* or *air-tubes* is much as in insects. It is through them that the air is conveyed to many of the tissues.

The *silk glands*. In the abdominal cavity, immediately over the spinnerets, the silk glands will be observed. Each gland is composed of a brush of tubular organs of which the more anterior are the larger.

The Nervous System. — The *brain* or *supra-œsophageal ganglion* occupies a position in front of the œsophagus and innervates the ocelli and the cheliceræ.[1] The *œsopha-*

[1] The arrangement of the ocelli is different in different species. In the present type two pairs of *median ocelli* form a quadrant, while a pair of lateral ocelli will be found on elevations just above the base of each chelicera.

geal collar connects the brain with a large *thoracic ganglionic mass* from which nerves extend to the lateral appendages and into the abdomen.

Finish the drawing already begun, representing the diaphragm, lung-sac, tracheæ, silk glands, and nervous system.

A LEPIDOPTEROUS INSECT

(*Sphinx sp.*).

LARVAL STAGE.

External Anatomy. — The body of the *larva* or *caterpillar* is elongated and vermiform and, like that of Arthropods already studied, is made up of serially arranged *segments* or *metameres*.

The *head* bears, anteriorly and laterally, a pair of large, convex *parietal plates* which meet above in the median line and enclose, lower down, a smaller, median, triangular *frontal plate*. The *ocelli* are found as a series of pigmented dots at the lower anterior portion of each parietal plate. How many ocelli are there?

The first pair of cephalic appendages, the *antennæ*, take their origin from the lower anterior angles of the parietal plates. Each antenna is made up of several joints.

The opening of the *mouth* will be found midway between the antennæ. It is bounded anteriorly by a two-lobed *upper-lip* or *labrum*, and laterally by a pair of strong chitinous *jaws* or *mandibles*. In what plane do the mandibles move?

The *maxillæ* lie immediately posterior to the mandibles. Each is provided with a small jointed organ, the *palpus*. In the median line between the maxillæ is the cone-shaped *lower-lip* or *labium*. It bears the external opening of the duct leading from the *silk-glands*.

Make drawings of the head as seen from the side and from below.

The segments immediately following the head, the *pro-*, *meso-*, and *meta-thoracic*, are each provided with a pair of jointed appendages, the *thoracic legs*.

Behind the thoracic region, the *abdomen* extends as a series of ten segments. Do the first two abdominal segments bear appendages? The fleshy appendages of the third, fourth, fifth, and sixth abdominal segments are called the *prolegs*. Note that each is terminated at its free end by a series of hooks. The seventh and eighth segments are not provided with appendages, though the eighth frequently bears a median dorsal spine. The ninth segment is short and somewhat suppressed, while the tenth is pierced by the *anus* and bears a pair of *anal prolegs*. In the embryo all the abdominal segments are provided with rudimentary appendages.

Extending along each side of the animal are a series of *spiracles*, openings which lead within the animal into an elaborate system of branched *respiratory tubes* or *tracheæ*. The spiracles do not occur on every segment, but are present upon the first thoracic and upon all the abdominal segments except the ninth and tenth.

Make a drawing of the animal as it appears when viewed from the side.

Internal Anatomy. — With a fine pair of scissors cut through the integument, along each side of the animal a little above the line of spiracles, from the side of the head to the ninth abdominal segment. Remove the dorsal piece without disturbing the underlying viscera.

The Circulatory System. — The *heart* will be observed as a median tube extending along the mid-dorsal line of the animal, immediately below the integument. The *blood*, which contains *amœboid corpuscles*, enters the organ through certain lateral *ostia*, and is forced anteriorly, where it enters the *aorta*, from whence it is distributed to the various sinuses of the body.

The Muscular System. — Many parallel bands of muscles, segmentally arranged, will be observed immediately under the integument. In the head they become specially developed in the neighborhood of the mouth parts.

Remove the heart and the laterally lying muscle bands. — The *body-cavity* will be found to be apparently filled with the subdivisions of the *fat-body*, though more careful examination with a lens will reveal the presence of other organs of somewhat the same appearance.[1]

The Digestive System. — The *mesenteron* or *stomach* will be observed as a large, rather thick-walled cylinder extending antero-posteriorly through the body-cavity. Followed anteriorly, the stomach is found to lead from a thinner-walled *œsophagus*. Posteriorly, the stomach becomes abruptly constricted and passes into the *intestine*, which in turns opens through the *rectum* to the exterior. The *salivary glands* will be found lying anteriorly, along the sides of the œsophagus. Their ducts open into the mouth. Associated with the salivary glands are the *silk glands* or *serictaria*, a pair of elongated organs which may be found lying side by side along the lower surface of the anterior portion of the mesenteron.[2]

[1] The substance of the fat-body is loose and flocculent.

[2] Both salivary and silk glands are elongated tubular organs. Each salivary gland extends as a coiled thread from the dorsal region of the

The external opening of the silk glands has already been noted.

Make a drawing of the alimentary tract.

The Excretory System. — A series of irregular tubes will be observed lying upon the posterior half of the mesenteron, each pursuing a somewhat irregular course and presenting, when seen under the lens, a beaded appearance. These organs are the *Malpighian tubes*, six in number. Each, after pursuing a tortuous course, empties into the intestine.

Make a drawing of the excretory system.

The Respiratory System. — The spiracular openings have already been noted. Each leads into a series of branching tubes, the *tracheæ*, which subdivide into very minute subdivisions, carrying the air to the most remote tissues. The tracheæ are lined with chitin and are strengthened by a spirally coiled chitinous ridge.

third thoracic or first abdominal segment along the lateral surface of the anterior portion of the digestive tract, in its course being partly covered by the lobes of the fat-body.

The *salivary duct* passes down the posterior surface of the bundle of muscles that works the jaws.

The silk glands are much larger than the salivary glands and occupy a very different position. Their ducts lie immediately below the œsophagus and the anterior portion of the stomach. The greater portion of each gland appears as a coiled cord, more or less surrounded by the fat-body and lying on a level with the spiracular openings, in the middle and posterior portions of the body-cavity.

The salivary and silk glands should not be confused with the much more delicate Malpighian tubes, which are arranged in more nearly parallel lines along the outer surface of the stomach.

The Reproductive System. — The *sexual glands* are somewhat difficult to find in alcoholic specimens. They lie in the fifth abdominal segment, immediately beneath the dorsal integument. In favorable specimens a delicate duct may be traced from each, around the intestine, to the lower side of the body-cavity.

The Nervous System. — The nervous system has the same general disposition that has already been noted for related Arthropods. The *brain* will be found far forward, immediately over the mouth, and uniting with the *infra-œsophageal* ganglion through the *circum-œsophageal collar.*

From the brain, which is divided into a right and a left half by a median furrow, nerves may be traced to the ocelli, antennæ, and to the œsophagus.

The *first* or *infra-œsophageal ganglion* innervates the mandibles, the labium, and the silk glands, and by its two connectives unites with the next following ganglia.

The *second ganglion* is of relatively small size. It innervates certain muscles of the head and neck and the first pair of legs.

The *third ganglion* innervates the second pair of legs, while the *fourth ganglion* supplies the third pair.

The *abdominal* ganglia are seven in number, — the first abdominal segment bearing the *fifth*, the second segment the *sixth*, the third segment the *seventh*, the fourth segment the *eighth*, the fifth the *ninth*, the sixth the *tenth*, and the seventh the *eleventh ganglion.* The eleventh ganglion is, moreover, clearly divided into an anterior and posterior half, and is, hence, probably composed of two fused ganglia.

There are no nerve-centres in the remaining abdominal segments.

While the connectives leading from the second to the third and from the third to the fourth ganglia are free and form loops, those leading between the remaining ganglia are more or less united together.

Make a drawing of the dorsal view of the nervous system.

AN ORTHOPTEROUS INSECT

(*Acridium americanum*).

ADULT STAGE.

External Anatomy. — Observe that the three divisions of the body, *head*, *thorax*, and *abdomen*, are here much more definitely outlined than they were in the caterpillar.

The head bears a pair of *antennæ*, each made up of several joints; a pair of large *compound eyes;* a median and two lateral *ocelli;* a bilobed *labrum* or *upper-lip;* a pair of strong *mandibles* or *jaws* which move laterally; a pair of *maxillæ* lying immediately behind the mandibles, and each bearing a lateral, jointed apparatus, the *palpus;* and a bilobed *lower-lip* or *labium*, which lies posterior to the maxillæ, and is also provided with palpi. As the mouth-parts are opened and the *mouth* exposed, the *tongue* will be noticed as a chitinous papilla on its floor.

The *thorax* has the same segments that were present in the caterpillar. The *prothorax* is the largest of the three and bears the first pair of *legs*. Its *tergum* or dorsal portion is enlarged and forms a shield. The *meso-* and *metathorax* are somewhat united together. The mesothorax bears the *second pair of legs* and the *first pair of wings*, while the metathorax bears the much larger *third pair of legs* and *second pair of wings*.

The legs will be found to consist of a series of joints and to be terminated by a pair of hook-like organs. The joints

of the legs are as follows: The *coxa*, short and ring-like, bears at its free end the *trochanter*, which is a little smaller than the coxa, to which, in the third leg, it becomes fused. The trochanter bears a long joint, the *femur*, which, at its free end, bears another long joint, the *tibia*. The *tarsus* is made up of a series of small movable joints which bear pads on their lower surfaces. The tarsus is terminated by the *claws*.

The *fore-wings* or *wing-covers* differ from the *true wings*. Note the arrangement of their *veins*, the rib-like lines which strengthen them and which are in communication with the circulatory and tracheal system. The second or *true-wings* are, when at rest, folded like a fan. Note the arrangement of the veins and see if the wing-covers, when expanded, do not support the anterior edge of the true-wings.

The *abdomen* is composed of a series of ten *segments* or *metameres*, all but the last two or three of which are very evident. There are no abdominal appendages.

The *first abdominal segment* is somewhat modified from its association with the metathorax. Laterally, it is pierced by two large openings, one on either side, which lead into the *auditory organs*. The abdominal segments of the two sexes from the first to the eighth closely resemble each other.

The *ninth abdominal segment of the female* is only about one-fourth as wide as the eighth, and is not provided with an independent sternal piece, the sternum of the eighth extending to the posterior end of the mid-ventral line. The posterior edge of the *tenth abdominal segment* follows a line parallel with posterior edge of the eighth. There project posteriorly from the tenth segment a series of more or less flexible pieces of which the dorsal is the larger and may

represent the tergum of an eleventh segment. This dorsal piece rests laterally upon a pair of triangular plates, the *podical plates*, the lower edges of which follow the course taken by the lower edges of the ninth and tenth segments. Resting laterally upon the podical plates are short pointed processes, the *cerci*. The *ovipositor* fills up that opening at the posterior end of the body, which is bounded by the free edges of the podical plates and the eighth sternal piece. The ovipositor is made up of two dorsal and two ventral pieces, all of which have sharp chitinous points. The *anus* opens between the dorsal pieces, and the *oviduct* between the ventral.

The *abdomen of the male* is relatively smaller than that of the female. The ninth and tenth segments are provided with a common sternal plate. The podical plates are reduced, while the cerci are relatively larger. A *subgenital* plate lies below the podical plates and continues posteriorly the ventral series of sterna.

Spiracles will be observed as follows: The most anterior is on the posterior edge of the prothorax, under the lateral fold of the shield-like piece. The second is at about the same level, behind the edge of the mesothorax. The third pierces the first abdominal ring just within the anterior margin of the auditory organ. The seven succeeding abdominal rings are each provided with spiracular openings. Spiracles do not occur on the ninth and tenth segments.

Internal Anatomy. — Carefully open the animal along the mid-dorsal line.

The Circulatory System. — The *heart* will be found occupying the same place that it has occupied in many other Arthropods, and presents no remarkable features.

The Muscular System. — Lying either side of the heart are the longitudinal *abdominal muscles.* The thorax contains several bundles of *alary muscles.* Are those which move the fore-wings separate from those which move the larger posterior wings? *Fat-bodies* may be found forming a loose tissue throughout the cavity of the abdomen.

The Respiratory System. — The external openings of the respiratory organs, or *tracheæ,* have been already noted. They will be found to lead, within the body-cavity, to a many-branched system of tubes which convey the air to the tissues, as has already been observed in the caterpillar.

The Reproductive System. — The *reproductive gland* rests in the upper part of the abdominal cavity. The *ovary* is a bilateral organ which is continued posteriorly into a pair of *oviducts.* The oviducts pass to the lower side of the abdomen, where they unite and form the *vagina.* The vagina opens to the exterior between the ventral pieces of the ovipositor. Slightly above and anterior to the external opening of the vagina, the much smaller opening which leads from the *bursa copulatrix* will be found. The bursa is a small sac which receives the spermatozoa. It is situated beneath the *rectum.*

The Digestive System. — The *œsophagus* leads into a large *crop* which extends from the mesothorax to the anterior part of the abdomen. Lying laterally to the crop are the branched *salivary glands,* the ducts of which may be traced to their openings into the mouth. In the abdomen the crop opens into the *stomach* which extends over half the length of the abdominal cavity. The stomach opens into the *intestine.* The *gastric cœca* are a series of pouches which lie parallel to

the stomach and open into it. How many are there? The intestine is somewhat less than the stomach in size. It receives the ducts which lead from the *Malpighian bodies*. The latter appear as a tangle of minute tubes. The intestine opens into the *rectum*, which in turn opens through the *anus* to the exterior.

The Nervous System. — The *brain* occupies the same position that it has been found to occupy in all the Arthropods that have been studied. The *œsophageal collar* and *ventral nerve-chain* present no new features. Is there a ganglion for each thoracic segment? There are five *abdominal ganglia*. In which segments do they rest?

A SIMPLE TUNICATE

(*Molgula manhattensis*).

THIS species is quite abundant along the New England shores, attached to submerged stones and woodwork. Though occasionally solitary, the individuals are more often found in clusters of varying sizes. It is our most common Simple Tunicate, and is related to the *Ciona* so frequently mentioned in the European text-books.

External Anatomy. — Though individual specimens vary, the more general shape of the present species is that of a two-necked flask, the larger neck or siphon serving for the entrance of water and food, the other, slightly smaller and often abruptly curved, serving for the escape of the strained water, the waste products of digestion and excretion, and also for the egress of the ova or embryos. The *incurrent opening* or *mouth* is surrounded by a series of *circum-oral lobes* or *tentacles*, which may be folded in such a way as to completely close the orifice. Are similar lobes arranged around the *excurrent* or *cloacal* opening? The outer surface of the animal has a felty appearance, due to the presence of innumerable *papillæ*. The papillæ entangle foreign matter, and thus the animal secures a protective coloring. In certain specimens the deeply colored viscera are to be seen through the semi-transparent body walls.

Note that the only plane that will divide the animal bilaterally passes through the long axis of each neck. Subsequent study will show that this plane actually separates the right from the left. The incurrent siphon arises from the morphological *anterior end*, while the cloacal tube extends from the *posterior end*. The shorter distance between the two is along the *mid-dorsal line*, while the greater distance marks the *ventral line*. Through the walls a light-colored band will be observed, extending along the anterior portion of the mid-ventral line. It is the *endostyle*, and, as subsequent dissections will show, lies along the floor of the *pharynx*.

The outer covering of the animal, "*tunic*" or "*test*," should now be partially removed from the left side. This covering will be found to be thick and tough: its outer surface, except where the specimen was attached to others, rough and dirty; its inner surface smooth and easily separated from the transparent underlying "*mantle*."

The entire tunic may now be removed, and its only points of firm attachment to the underlying mantle are found at the margins of the incurrent and excurrent openings. Through the transparent mantle the contained deeply colored viscera are to be seen, while the mantle itself is found to be provided with certain clear white bands of *muscle tissue*. These *muscle bands* are especially abundant on the tubes, where they are arranged in *longitudinal bundles* and *transverse fibres*. How are they arranged on other parts of the mantle? It is through the activity of these muscles that the animals contract and forcibly eject the contained sea-water. The transverse fibres form *sphincters* around the mouth and at the base of each siphon, and move the six oral and four anal flaps.

Internal Anatomy. — Viewing the specimen from the left side, the course of the S-shaped *digestive tract* may readily be followed. The tract is made up of two parallel portions, the *œsophagus* and *stomach* forming the lower limb, and the *intestine* forming the upper. Lying just above the, often dark-colored, intestinal loop is the light-colored *sexual gland.* The delicate *sexual duct* extends dorsally and posteriorly, parallel with the posterior section of the intestine.

Lying deeper than the just mentioned viscera are six nearly parallel light-colored bands. They are the *branchial folds*, and will be considered later on.

At a point on the dorsal surface, about midway between the siphons, the *ganglion* will be observed as a small opaque spot. Notice the *nerves*, and, if possible, trace them to the siphon tubes.

Again follow the course of the opaque endostyle. It extends along the mid-ventral line, from near the base of the oral siphon to a point removed nearly 180°. Its posterior termination is near the point of origin of the œsophagus.[1]

Through the transparent mantle of the right side six branchial folds and a large sexual gland will be at once recognized. The duct leading from the sexual gland extends in a nearly horizontal direction towards the cloacal siphon. It often contains eggs or embryos. Lying parallel to and below the sexual gland, enclosed in a pericardium, is the *heart.* (In alcoholic specimens the circulatory system is not easily dissected.) Below (ventral to) the heart is a crescentic sac of considerable size. It is the *renal organ*, and often contains brownish flakes and concretions.

[1] The endostyle may be the homologue of the *hypobranchial groove* of Amphioxus, and of the *thyroid gland* of higher Vertebrates.

If the specimen is viewed from the dorsal side, a circular line will be found to extend from the anterior end of the endostyle around either side of the oral siphon to the ganglion. This line marks the position of the *peripharyngeal bands.*

Drawings of the right and left side of the animal should now be made.

By gently pressing upon the mantle it will be shown that there is a space between it and the deeper lying branchial bands. This space is the *atrium* or *peribranchial chamber,* and can be beautifully seen if one now carefully removes the cloacal siphon by cutting around its base, beginning just above the ganglion. The cavity thus opened is actually the *cloacal chamber,* though the right and left halves of the peribranchial chambers are seen to lead directly from it as we look down through the artificial opening. The branchial folds are now seen to form the longitudinal ribs of a reticulated sac, the *branchial sac* or *pharynx.* Crossing the bands at right angles are certain *transverse vessels,* and stretched between vessels and ribs is a delicate membrane pierced by minute openings or *stigmata.*

On the branchial sac a dorso-median line will be observed to extend from near the ganglion to near the posterior end of the endostyle. The *anal opening* will be found near the lower end of this line. The *intestine* lies along the floor of the left peribranchial chamber, and is often deeply colored from the presence of contained fæcal matter. The *openings of the genital ducts* are to be seen on the right and left external branchial walls respectively. The cloacal chamber receives then the waste products of digestion, the secretion of the sexual glands, and also the water which flows into it directly through the stigmata of the posterior wall of the

branchial sac as well as the water which flows from the two large laterally lying peribranchial chambers.

Slit the mantle from the opening already made, along the upper edge of the left peribranchial cavity and thence ventrally along a line midway between the endostyle and the lower loop of the digestive tract, cutting through the strands or *trabeculæ* that connect the mantle with the pharyngeal sac. The external wall may be now lifted and turned down and the extent of the left branchial chamber definitely established. On its external wall the exact course of the alimentary tract will be noted from the origin of the œsophagus at the point where the branchial bands converge, to the anus. Again note the transverse vessels and stigmata.

In the same way as above directed open the right peribranchial chamber and note the course taken by the sexual gland and renal organ. The two peribranchial chambers are not confluent along the ventral line.

Passing the point of a fine pair of scissors into the oral siphon, carefully cut along near the ventral line of the branchial sac midway between the endostyle and the ventral branchial band. The *pharyngeal cavity* will thus be opened. The twelve branchial folds converge from their origin, just below the peripharyngeal bands, to the opening of the œsophagus. Each lateral half of the *peripharyngeal bands*, taking its origin from the anterior end of the endostyle, passes dorsally to meet its fellow in the mid-dorsal line just back of the ganglion. Thus united, the bands continue posteriorly to the opening of the œsophagus as the *dorsal lamina*.

The endostyle now appears as a gutter-like structure, extending the entire length of the ventral side of the pharyngeal chamber. A circle of *branched tentacles* form a crown

around the siphonal canal, just outside the peripharyngeal bands.

Drawings should now be made of the structures thus shown on the interior of the pharynx.

Carefully remove the ganglion together with the immediately surrounding tissues, and examine it carefully in a watch glass.

From its inner surface a funnel-shaped process will be observed. This is the *dorsal tubercle*, and is a part of a small glandular mass, the neural gland. The course of the nerves can be readily determined by the adoption of the xylol method described for the nervous system of the Phyllopod Crustacea.

Make a drawing of the ganglion and of its associated structures.

General Remarks. — The outer covering of the Tunicates is variously described as the test, tunic, outer mantle, or sac. It varies considerably in different types, being in some delicate and transparent, in others tough and opaque. It is produced by the activity of a lining layer of epithelial cells, and is consequently comparable with the covering of many Invertebrates. It is peculiar in that it contains cellulose. The mantle or muscular sac (integument, second or inner muscular tunic) is also subject to considerable variation. In life it is attached throughout to the inner surface of the tunic, though in alcoholic specimens it is often partially free.

The Digestive System. — Food that is drawn into the pharyngeal sac by the activity of the cilia of the pharyngeal walls is collected by the mucus secreted, probably by the endostyle or by the sub-neural gland, and is carried along

the peripharyngeal bands to the dorsal lamina, and thence posteriorly along the "roof" of the pharyngeal sac to the opening into the œsophagus. In certain species the œsophagus opens into an enlarged stomach, and frequently accessory digestive glands are present. A median fold, the *typhlosole*, partially divides the lumen of the intestine.

The Excretory System. — Besides the renal organ already noted, there are certain small anomalous collections of vesicles around the intestine and in the tunic that are supposed to perform excretory functions. The neural gland is also supposed by some to function as a renal organ.

The Circulatory System. — In the Tunicata there are no true arteries or veins. The heart is a sac-like organ which forces the blood intermittently towards the posterior dorsal portion of the animal, and then in the opposite direction towards the anterior ventral. It is surrounded by a fold, the *pericardium*. During the *respiratory circulation*, a *ventral trunk* conveys the blood from the heart along the ventral portion of the animal, below the endostyle, and gives off lateral branches to the *transverse vessels* of the branchial sac. From these vessels the blood passes into the fine, irregularly disposed interstigmatic vessels, the meshes of which surround the stigmata. The *longitudinal* vessels in *Molgula* are arranged on the branchial folds already noted.

When the current is from the ventral towards the dorsal portion of the animal, the blood passes through certain vessels to the mantle, the digestive and reproductive organs. The blood from these organs is then collected in a *dorsal trunk*, which lies along the mid-dorsal line of the branchial sac, and from thence it passes into the transverse vessels. There are then, during this cycle, three systems. 1st, *Branchio-*

cardiac, conveying pure blood to the heart along the ventral trunk; 2d, *Cardio-splanchnic*, conveying blood from the heart to the viscera and other parts of the body; 3d, *Splanchno-branchial*, conveying impure blood from the viscera, etc., through the dorsal trunk to the branchial sac.

The test is supplied with blood through vessels that leave the main trunks near the heart.

The Nervous System. — The nerves from the ganglion may be traced to certain *sense-organs*. The *ocelli* are situated around the oral and cloacal orifices. The *tentacles* have already been noted.[1]

The Reproductive System. — The Tunicata are hermaphrodite, though probably not self-fertilizing, as the ova often reach maturity much earlier than the spermatozoa. In *Molgula* the ovary and testis unite and form a pair of hermaphrodite glands; in other forms the glands may be separate. Reproduction by budding is not infrequent. This process may give rise to small colonies, the individuals of which may have a common circulatory system. Such Tunicates are called Social in distinction from the Solitary Tunicates, of which *Molgula* is a type.

[1] For a long time the dorsal tubercle was supposed to function as a sense-organ, and was called the *olfactory tubercle*. It is now known to be the funnel-shaped extremity of a ciliated duct, leading from the neural gland. It has been compared to the pituitary body of Vertebrates.

APPENDIX.

A.

THE USE OF THE LABORATORY AND LABORATORY APPARATUS.

OBSERVANCE of the following regulations will facilitate laboratory work: —

During laboratory hours work, walk, and talk quietly, that your co-laborers may not be annoyed.

Handle microscopes and other instruments with care, keeping them clean and in order, and always, after using, return them to their proper places.

Instruments that are the property of the laboratory should be used with especial care, and never retained longer than is necessary for the immediate work in hand. On returning the same, be sure that it is to the proper place.

Reagents should be used sparingly, corks or stoppers returned to the bottles to which they belong, and the bottles returned to their proper places. Never pour back into the "stock bottle" any reagent that has been once made use of. Take care that your hands do not soil the label of the bottle. (It is well to wipe the lip of the bottle before and after pouring out the liquid.)

After each day's work, clear up your table and return everything that has been borrowed.

All organic refuse should be placed in the receptacle that has been provided for the same, and as free from liquid as possible.

Pour nothing but liquid into the sinks. Be especially careful that sand and shreds of flesh are not left in the sinks.

After using laboratory sponges, rinse them out and leave them in their racks. Never use them for absorbing grease or oil.

Paraffin chips or wax should not be allowed to fall on the floor.

Your own books, and certainly laboratory books, should not be handled with soiled fingers.

Keep the lockers, drawers, etc., that have been assigned to you in an orderly condition.

Finally, if you are so unfortunate as to injure laboratory instruments, books, or other property, inform the instructor of the extent of damage done.

B.

LIST OF INSTRUMENTS AND REAGENTS.

[Those marked with an asterisk should be owned by the student.]

* Large scalpel.
* Small scalpel.
* Large tweezers, with serrated points.
* Small tweezers, with smooth points.
* Large scissors, with "lock" joint.
* Small scissors, with fine points.
* Vial of entomological pins.
* Pair of needle holders and assorted needles.
* Small package of bristles, white and black.
* Hand lens or, better, a dissecting microscope.
* Assortment of small brushes.
* Drawing pencils, one hard and one medium.
* Note book.
* Erasing rubber.
* Bristol drawing-board.
* Slides and covers for microscopic work.

GLASS WARE.

* Several small and one large pipette.

Water bottle.

Assortment of heavy and light watch-glasses.

Spirit-lamp.

REAGENTS.

50% alcohol.
70% alcohol.
95% alcohol.
100% alcohol.
Acidulated alcohol.
Corrosive sublimate.
Perenyi's fluid.
Picro-sulphuric acid.
10% nitric acid.
Aceto-carmine.
Borax carmine.
Alum cochineal.
Alcohol carmine.
Turpentine.
Chloroform.
Chloroform and paraffin.
Collodion.
Collodion and clove oil fixative.
Clove oil.
Cedar oil.
Thyme oil.
Canada balsam.
Glycerine.
Vaseline.

C.

MICROSCOPES.

THE microscopes required for ordinary biological work should be "simple" and "compound."

The *simple microscope* may be either a pocket lens or one of the more elaborate dissecting microscopes figured in catalogues of optical instruments, etc. Pocket lenses of a very convenient form are made in German silver mountings. For laboratory work each lens should be provided with a long-arm support, that it may be held, if need be, over a dissecting dish, allowing the free use of both the operator's hands.

The compound microscope should be provided with eye-pieces and objectives that will give a magnifying power ranging from fifty to six or seven hundred diameters. The mechanical and optical parts of the microscope, and their uses, should be familiar to the student. The more important parts are as follows: The heavy foot, or *base*, often made in the shape of a horseshoe; the upright *pillar*, which may be supplied with a *joint*, allowing inclination of the instrument. The *stage*, generally black in color, is the horizontal support upon which the object to be examined is placed. The stage is often provided with a pair of *compressors* or *clips*, which need not be used in ordinary work. Immediately under the stage, and a part of it, is the *sub-stage*. The sub-stage is generally provided with a perforated apparatus — the *diaphragm* — for limiting the amount of light. In the more expensive instruments the sub-stage has also *condensing glasses* for increasing the intensity of the light. The *mirror* is provided with a plane and a concave surface. Though a more simple part of the instrument, the

mirror is most important. Successful observation frequently depends on the origin and intensity of the light and also upon the direction from which the mirror throws light upon the object. The *arm* of the microscope, reaching out from the pillar, supports more or less directly the *tube*, which in turn carries, partly within itself, the *draw-tube*. The tube may be moved up and down either by a rack and pinion — "*coarse adjustment*" — or by sliding easily through a *collar* supported by the arm. More delicate motion is secured by the use of the "*fine adjustment*," a small milled wheel, generally placed at the apex of the pillar.

The parts of the microscope thus far mentioned are generally included in the term "microscope stand." The optical parts consist of a series of *eye-pieces* or *oculars*, which fit into the upper part of the draw-tube, and a series of much more expensive *objectives* which screw into the lower end of the tube. A very convenient, though not essential, apparatus is the *triple nose-piece*, by means of which *three* objectives, revolving around a common centre, may be attached to the lower end of the tube, permitting an instantaneous change of magnifying powers. A little careful experimenting with the eye-pieces and objectives, under the direction of the instructor, will soon show their relative values as magnifying agents. One rule for adjusting the *focus* has no exceptions. "*Always* place the front of the objective near the object before looking in the ocular, and then move the tube *upward* in focussing." By observing this rule the objective will not be ruined by being forced into the object.

Objects may be examined as they float in watch-crystals, or they may be mounted on *slides* and covered with *cover-glasses*.

In case objectives or oculars become soiled, it is well to

call the attention of the instructor. An objective may be ruined by being wiped with a dirty cloth. If, through accident, fluid runs on to the stage of the microscope, be sure that it is *all* carefully removed from around the sub-stage. Be especially careful of acids, and do not let them long stand under the objective. Never wet the mirror. Do not allow the instrument to stand in strong sunlight. Always dust and put the instrument away after using.

D.

DIRECTIONS FOR DISSECTION.

VERY small objects are studied by "teasing," which consists of gently tearing them apart under a lens, or of placing them under a cover-glass and tapping the cover lightly with a needle, the result being observed under the compound microscope. All teasing should be done while the object is covered with liquid, generally water, glycerine, or alcohol.

With somewhat larger animals the small scissors, scalpel, and tweezers may be used, the object being pinned out under water. As the water becomes cloudy it should be renewed, or the cloudy portions may be removed with the large bulb-pipette.

It is only the larger animals that are ordinarily dissected without being flooded with water. The sponge should be freely used and, if possible, the animal should be frequently placed under the tap.

Nerves may generally be more easily separated from the

surrounding tissue if the specimen is allowed to stand for two or three days in 10% nitric acid. The acid, however, injures the other tissues.

Muscles are generally more easily studied in alcoholic specimens. Small blood-vessels should be injected either with a pipette or with a hypodermic syringe. The use of strong acetic acid will frequently aid in separating organs that are firmly held together with connective tissue.

Xylol may at times be advantageously used with small objects that have been preserved in strong alcohol. The xylol frequently clears the object in a most instructive way, presenting in the process one organ-system after another.[1]

Glycerine may be advantageously used as a teasing medium with many objects. Fragments of tissue thus isolated may be kept under the cover-glass for a considerable time.

Schneider's aceto-carmine is a penetrating stain that can be used with fresh tissues, either during the process of teasing or after the objects are under the cover-glass. Material thus stained may be washed in water and kept for a short time by being mounted in glycerine.

[1] See page 83.

E.

METHODS OF KILLING, FIXING, AND HARDENING.[1]

UNLESS animals or tissues are killed by some one of the recognized agents, they will not give the best histological results and the organs themselves will be often distorted and unnatural. The killing agents that are more ordinarily used are the following: Hot water (80° C.), weak alcohol, corrosive sublimate in hot or cold saturate solution, acetic acid, Kleinenberg's picro-sulphuric acid, Perenyi's fluid, and the varying chromic solutions.

While the above reagents may be successfully used with many animals, there are nevertheless many organisms that violently contract on coming in contact with them. Such organisms must first be rendered insensible by being anæsthetized and then plunged into the killing agent.

Chloral hydrate, weak alcohol, cocaine, chloroform, hydroxylamine, etc., have been used as anæsthetizing agents.

Objects that have been killed by the use of many of the above-mentioned agents are still soft and flabby and would, if allowed to remain for any considerable length of time, disintegrate. Several so-called "fixing" and "hardening agents" have been adopted which are also frequently the same as are adopted for killing. Such are corrosive sublimate, picro-sulphuric acid, Perenyi's fluid, and the chromic solutions. Alcohol, used in regularly increasing grades of

[1] The methods of killing, fixing, hardening, preserving, staining, etc., are only given in the briefest possible way. It is taken for granted that the students have at hand either an instructor or the admirable text-books of microscopical technique which have been written by Dr. C. O. Whitman and A. B. Lee.

strength from 50 to 95%, is also frequently used as a fixing and hardening agent. To these might be added osmic acid and cupric sulphate.

The length of time that objects should be allowed to remain in the fixing fluids varies according to the fluid used and the tissue or animal being treated. As a general rule, a large quantity of fluid, two or three times the bulk of the object, should be used, and this renewed as soon as it becomes cloudy.

F.

PRESERVATION.

OBJECTS that have been sufficiently fixed or hardened are thoroughly washed in water, excepting such as have been treated with picro-sulphuric acid or alcohol, and placed in 70% alcohol. An abundance of alcohol should always be used. Picro-sulphuric preparations should be repeatedly washed in alcohol of 70% before being finally placed in the cabinet.

G.

METHODS OF STAINING.

ORGANISMS that when living are brightly colored are generally bleached by the action of the fixing or preserving media, and tissues which are really very dissimilar in structure not infrequently appear to be quite similar. To reveal these masked or hidden structures, microscopists have made use of certain stains.

Stains are either diffuse or selective. The former *dye* the tissues throughout, while the latter stain only certain parts of

the tissue and leave other parts unaffected. Selective stains are for this reason spoken of as "differential stains." Certain tissues will absorb a certain color in a certain fixed way and may be quite unaffected by another stain.

The stains more commonly used are made from carmine, cochineal, hæmatoxylin, or from some of the numerous aniline dyes.

Stains are also either aqueous or alcoholic. Alcoholic specimens may be placed directly into alcoholic stain, but should be first washed with water before being placed in aqueous stain. The length of time that an object requires for proper staining depends on the object, the way it has been hardened, the length of time it has been preserved, and the stain that is adopted.

The objects are taken from the alcoholic stains, washed in acidulated alcohol for a short time, and then placed, until wanted, in 70–90% alcohol. If an aqueous stain has been used, the excess of stain is washed out with water, often acidulated, and then the object is placed successively in 50, 70, and 90% alcohol.

H.

MOUNTING.

MANY small organisms, and portions of larger, may be mounted on glass slides and retained, if properly prepared, indefinitely. The mounting medium most commonly used is Canada balsam. The order of procedure is as follows: —

The object, stained or unstained, is carried up through the grades of alcohol, and is finally placed in absolute alcohol, that the last trace of water may be withdrawn from it. This is the process of *dehydrating*.

After dehydrating, the object is *cleared, i.e.*, an oil, which will mix with alcohol on the one hand and with Canada balsam on the other, is allowed to take the place of the absolute alcohol. This change for all small objects may be made by lifting the object from the alcohol and, after draining off the excess of alcohol, placing the specimen for a few minutes in oil of clove, or oil of cedar, or turpentine, or organum oil. After remaining in the oil for a few moments, the object will become quite transparent. It is now ready to be placed on the slide and, after the oil is drained off, covered with a drop of Canada balsam.

A clean cover-glass is now warmed for a moment over the spirit-lamp and allowed to gently rest on the balsam and object. If there is an excess of balsam, it may be removed with a cloth dampened with turpentine. If there is not a sufficient quantity of balsam, a drop may be placed on the slide near the edge of the cover, when it will run under by capillary attraction.

If the object is rather thick and does not well support the cover-glass, the latter may be supported with rubber or glass rings made for the purpose, or with small pieces of a broken slide. "Wax feet" have been recommended by Kukenthal.[1]

I.

METHODS OF IMBEDDING AND SECTION CUTTING.

THOUGH sections of certain tissues may be cut without previous preparation, the best results are to be obtained by the adoption of one of the following methods: —

[1] To three parts of wax are added one part of vaseline and one part of Canada balsam.

The Paraffin Method. — The object, which should be of small size, not often exceeding 1 cu. cm., having been stained, is thoroughly dehydrated as previously directed.

From absolute alcohol the object is to be placed in some fluid that will mix on the one hand with alcohol and on the other with paraffin. Such fluids are oil of cedar, oil of clove, oil of creosote, turpentine, chloroform, etc. Immersion for two hours in any one of these will often be sufficient.

Flakes of paraffin are now whittled into the oil, or chloroform, until the point of saturation is reached, and the specimen allowed to stand for an hour or longer. If the fluid is warm, a less time may suffice.

The object is now taken from the saturated oil or chloroform, and placed in melted paraffin, the temperature of which should not exceed 35° C. In from one to two hours' time the object will be thoroughly infiltrated.

Make now a paper box, somewhat larger than the object, and by means of a warm pipette fill the box with paraffin, and place the object therein, noting the position in which it lies.

In a few moments a film will have formed on the surface of the paraffin, when the box may be placed in a glass of water until thoroughly cold.

Remove the box, wipe dry, and with a sharp knife trim off the paper from around the block of paraffin. Bearing in mind the position of the object when dropped into the box, scratch on the block a line that will designate the plane in which the object is to be sectionized.

If the object is not to be immediately cut, it should be properly labelled and placed away for safe-keeping.

Objects imbedded in paraffin *may* be cut, by a steady hand, with no other instrument than a sharp razor. Of late years, however, some form of mechanical section-cutter — *microtome* — is to be found in every biological laboratory. Such microtomes are the Minot, which is the best for paraffin work, and the Thoma, which may be used for paraffin or for celloidin.

If paraffin has been used as an imbedding mass, the object-holder, metal or wood, is warmed, and the block attached by cool water being allowed to run over block and holder until a firm union has been obtained. The position of the object in relation to the cutting-plane of the knife has, of course, been observed.

Adjust the paraffin block, and cut off thin sections of paraffin until the neighborhood of the object is reached. Trim the remaining portion of the block so that it will be in the form of a cube, the edges of which are parallel to or at right angles with the cutting edge of the knife. Adjusting the gauge so that thin sections of equal thickness will be cut, proceed until as many slices are secured as are needed.

The following annoyances may arise: —

1st. The sections roll. — Correct, by cutting thinner sections, or by using softer paraffin, or by cutting in a warmer room. Sections sometimes roll when the knife is dull.

2d. As the knife passes through the object, the tissue appears granular and the section porous. — The trouble is either that the imbedding was not properly done or the knife is not properly sharpened.

3d. The block at its upper portion becomes white and broken. — See if the cutting edge is not set so that it is further from the block than the back part of the knife.

4th. The sections crimp, the section of paraffin being

somewhat smaller in area than the block from which it is cut. — The sections are too thin, or the room is too warm, or, quite likely, the knife has become smeared with dirt or paraffin. Clean the knife on both sides by stroking it from the back toward the edge with a clean cloth dampened with chloroform.

The Celloidin Method of Imbedding and Cutting. — The object is first stained *in toto*, dehydrated, infiltrated with thin, medium, and thick celloidin or collodion, and finally placed in a paper tray filled with the thick collodion. In a few minutes a film will form over the exposed surface of the collodion, when the paper tray and its contents are placed in a jar of strong chloroform, in which, after a few hours, the collodion becomes quite hard.

The tray is now taken from the chloroform and, after the paper has been removed from the hardened block, the collodion with its enclosed object is placed in a vial of white oil of thyme or some similar oil. If the block of collodion is not large, in a few hours it will become as clear as glass, the stained object appearing as if suspended in a transparent fluid.

For the process of orienting, the block of collodion may be taken from the oil, placed in a watch-crystal, and, after covering with the oil of thyme, examined with a lens or, if very small, with a compound microscope. The side of the block that is to be attached to the object-holder of the microtome is now selected, wiped dry of the oil, and immersed for a moment in ether, and then smeared with thick collodion. The object-holder, a block of wood rather than cork, is smeared in the same way, and the two collodionized surfaces are brought together.

The holder and collodion block are now immersed for a few minutes in chloroform, or long enough for them to become firmly united.

The object-holder is finally screwed between the jaws of the microtome, and covered, by means of a camel's-hair brush, with oil of thyme. The microtome knife is flooded with the same oil.

After a few sections have been cut from the block of collodion, the relative position of the plane of the knife to the axis of the object can be definitely established, and the object definitely oriented.

The sections are now cut and may be at once transferred to the slide, covered with balsam, and mounted; or if they are not immediately needed they may be kept indefinitely, together with the block, in a vial of thyme oil.

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